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MEAT IN NUTRITION. XVIII. GLYCOGEN IN MATERNAL AND FETAL LIVERS OF RATS FED A DIET CONTAINING DRIED AUTOCLAVED PORK MUSCLE

by

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INTRODUCTION AND PURPOSE OF EXPERIMENT

Because of the important role played by pork in the economic life of the people of the state of Iowa and the relatively high daily consumption of pork by the farm families of that state, the Nutrition Laboratory of the Foods and Nutrition Department of Iowa State College has been interested in investigating the dietary value of pork.

At the initiation of this study a diet was formulated, supposedly adequate in the then known dietary essentials, and synthetic except for the protein which was supplied by autoclaved, dried pork muscle. The effect of this dietary regime upon the well-being of the albino rat has been studied in the Iowa State College Laboratory by several investigators over a period of about ten years. Since an adequate diet must provide for successful reproduction and lactation and support the life functions over successive generations, as well as to promote maintenance and growth, interest soon centered on the effects of such a diet upon the reproductive performance of the rat and the continuance of the species.

One of the first studies reported was that of Dyar (1935) who found that not only was the growth rate of the

rats fed the pork diet retarded, but that there were definite abnormalities of the reproductive functions. Most striking, perhaps, was the fact that all of the rats of the second generation receiving pork were sterile, as were 70 per cent of the first generation. Dyar also observed a lack of success in parturition and lactation. Further studies were made by King (1936), Wilcox (1937), Walliker (1938), Armstrong (1939), and Campbell (1940) in attempts to discover the etiology of the defects observed and to describe and study the metabolic derangements caused by the pork diet.

Some of the abnormalities observed have been a lengthening of the oestrous cycle, a prolongation of the gestation period, partial or complete gestational failure, and an inability on the part of the mother to suckle her young. The most interesting effect observed has been the death of mothers at the time of parturition. During the course of Dyar's investigation (1935), 33 per cent of the pork-fed mothers died. The symptoms exhibited by the females preceding death are fairly uniform and closely resemble those described as typical of the toxemias of pregnancy in man, sheep, and rabbits. The rats appear normal until the close of gestation when they become limp, lethargic, and cold to the touch, with pale ears

and paws. Often the condition is preceded by the appearance of bloody urine, which is not, however, an unfailing sign of approaching illness.

During the acute phases of the disturbance the rats show evidences of extreme discomfort, as exhibited by a clenching of the teeth and failure to support the head, which is often rested on the food cup or pressed into a corner. Vaginal hemorrhage is a common occurrence and death is often preceded by violent convulsions. At times none of the earlier symptoms are observed and the rat appears normal until the dramatic and sudden onset of a convulsion, culminating in death.

Upon autopsy certain pathological changes are evident. The livers are yellow, swollen in appearance and often extremely friable. The kidneys are enlarged and gorged with blood. The feti are usually found dead. Sometimes the feti appear macerated or even partially decomposed. Hemolysis of fetal blood and thrombi in the umbilical veins were noted by Armstrong (1939). Since the liver exhibited the most obvious gross abnormalities, both histological and chemical examinations of that organ were made. Histological studies (Armstrong, 1939) have shown fatty infiltration and fatty degeneration of the liver cells of pregnant pork-fed rats and chemical

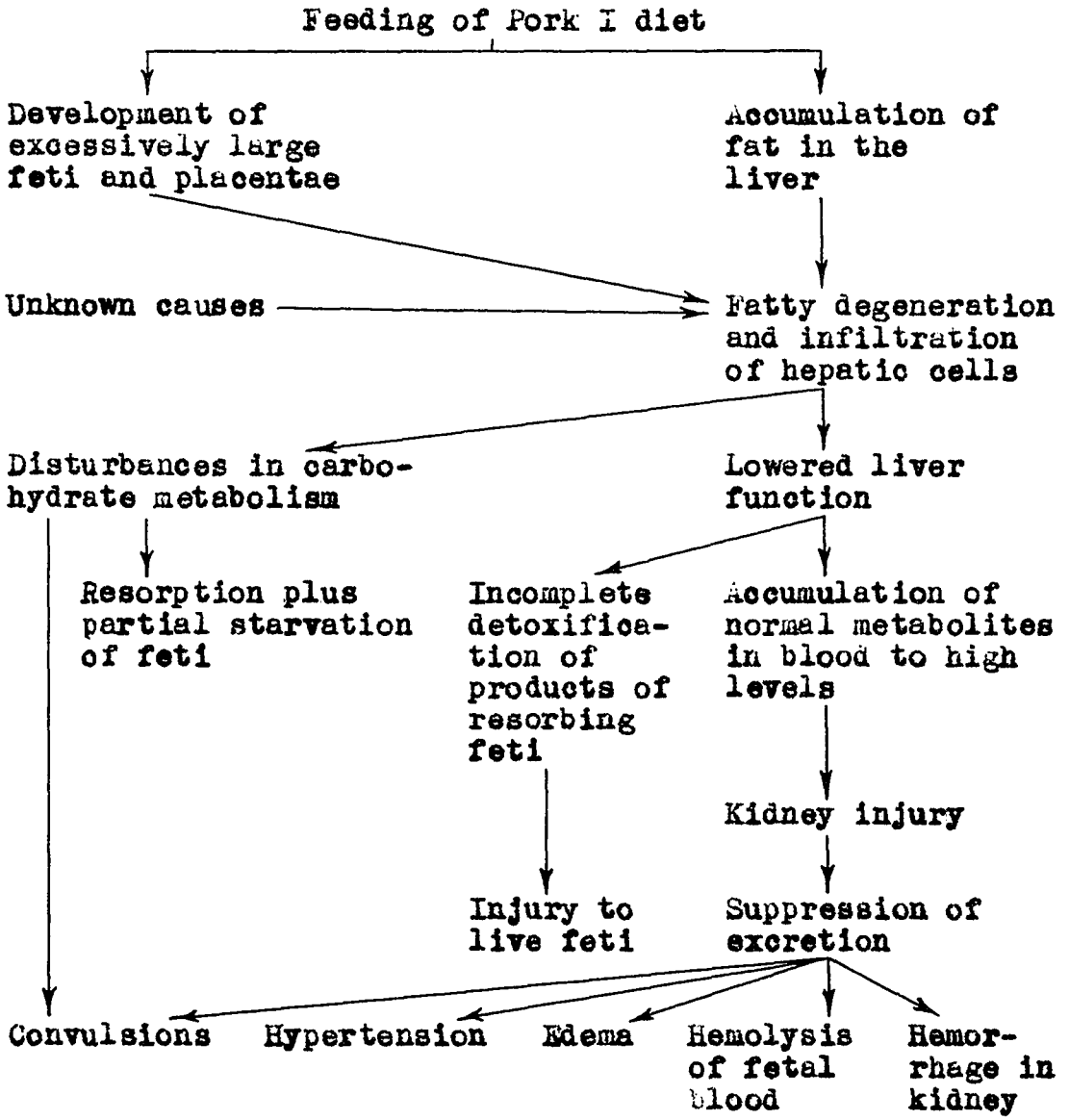
analyses have shown a high liver fat content. The average per cent of liver fat calculated on the dry basis was approximately 31 per cent for the animals that died and 39 per cent for the non-moribund pork-fed rats in contrast with 22 per cent for normal, pregnant controls.

As early as 1902, Rosenfeld discussed the "antagonism of fat and glycogen in the liver". He indicated that fattening with carbohydrates brings about an abundant storage of glycogen in the liver and that the liver cells thus enriched may refuse to take on fat. Rosenfeld made the interesting observation that sea animals, such as the fish and the whale, that consume mostly fat and protein nutrients have enormously fat livers. The pork diet fed in the present study is one that contains about 20 per cent of fat and is apparently conducive to the production of fatty livers. Conversely to Rosenfeld's statement concerning the refusal of the glycogen-rich liver cells to take on fat might not the opposite be true? Do liver cells loaded with fat refuse to take on glycogen? If this does occur, are derangements in carboyhydrate metabolism associated with the pregnancy disease? Armstrong has suggested certain of these relationships in an interesting diagrammatic representation of the theoretical sequence of events resulting in the pregnancy disorder.

This diagram is reproduced as Figure 1 and indicates that disturbances in carbohydrate metabolism may result from the fatty degeneration and infiltration of hepatic cells. Inadequate carbohydrate stores may lead to resorption and starvation of feti or to convulsions and other symptoms observed in the sick rats. Another theory might be that a lack of circulating glucose may result in placental derangements and the consequent production of toxic substances.

Cori (1926) has devised a plan to study carbohydrate metabolism by a balance technique which involves analyses pertaining to absorption, glycogen in liver and muscle, glucose in blood and urine, and the respiratory quotient. Since it was impossible to attack the present problem on such a wide scale, it was felt that if a defect existed it might be reflected first in the concentration of liver glycogen. Therefore, the present study of the concentration of glycogen in the liver was undertaken to test the hypothesis that consumption of the pork diet may lead, either directly or indirectly, to a derangement of carbohydrate metabolism.

FIGURE 1. THEORETICAL REPRESENTATION OF SEQUENCE OF EVENTS RESULTING IN ACUTE PREGNANCY DISORDER[1]



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1. Armstrong, W. E.
1939. Meat in nutrition. XV. Certain characteristics of gestational performance in albino rats fed a diet containing dried autoclaved pork muscle. Unpublished Thesis, Ph.D., Library, Iowa State College, Ames, Iowa.

PLAN OF EXPERIMENT

Whether the fatty livers consistently observed in pregnant rats maintained on a diet containing dried, autoclaved pork muscle as the source of protein are associated with any disturbances in carbohydrate metabolism has been studied in the present investigation. The quantity of glycogen deposited in the liver under standardized conditions of feeding was chosen as an index of the ability of the rat to metabolize carbohydrate.

Five series of rats have been studied. In Series I, the quantity of glycogen present in the liver of pregnant rats fed the pork-containing ration was compared with that present in pregnant control rats maintained on the standard diet of the stock colony and with that present in virgin rats of the same age reared on the experimental diet and on the diet containing pork muscle. The analyses of the livers of the pregnant rats were made on the 21.5 day of the gestation period. After a starvation period of 13 hours, each animal was offered 4 gm. of the ration upon which she had been reared. The liver was removed for analysis after seven hours.

It seemed possible that the quality of the food given after the starvation period might influence the

amount of liver glycogen deposited. Therefore, another group of starved pregnant females reared on the pork diet was fed 4 gm. of the stock diet seven hours before removal of tissues. This group comprised Series II. Findings were compared with those of the pregnant rats fed pork in Series I.

It was thought that the amount of glycogen deposited by the mother in the fetal livers might prove to be of interest. Consequently, the livers of the feti from part of the pregnant females in Series I and Series II were removed and analyzed.

In order to rule out variations in the quantity and quality of food given after the starvation period, a more carefully controlled experiment was designed as Series III. In this experiment, measured amounts of a standard glucose solution were given by means of a stomach tube to groups of starved pregnant and virgin rats reared on the experimental and control diets. Liver glycogen and the reducing contents of the gastrointestinal tract were determined. Thus, information was obtained on the absorption of glucose from the intestinal tract, as well as the deposition of liver glycogen. In this test it was also necessary to determine the average amount of preformed glycogen in the liver, and the normal reduction values of the contents of the empty gastrointestinal

tract. These analyses were made after a 13-hour fast on groups of pregnant and virgin rats on both the experimental and stock diets.

As Armstrong (1939) has indicated, only about 30 to 40 per cent of the pork-fed rats develop the toxic symptoms. We were interested in determining whether there was any difference in glycogen metabolism in rats that developed the pregnancy disease and those that did not. Since the acute symptoms usually appeared very late in the gestation period, it seemed possible that the glycogen determinations in the preceding series were made too long before parturition to catch a break-down of glycogen, if that phenomenon did occur. Therefore, an effort was made to analyze the livers of some pork-fed rats on the last day of parturition. In Series IV, a group of fasted "pork rats" was fed a measured amount of a glucose solution by stomach tube on the twenty-second day of gestation. If parturition occurred before the elapse of the four hour digestion period, the rat was discarded.

The original plan of the study excluded the observation of toxic symptoms since, as explained previously, these symptoms usually occur on the twenty-second or twenty-third day of gestation. It seemed important, however, to learn something regarding glycogen deposition

in a rat, definitely ill. Therefore, a small group of rats that exhibited toxic symptoms, such as bloody urine, pale ears and paws, and drop in body temperature, was studied in Series V. Obviously, in this series, pre-experimental conditions could not be controlled.

Steenbock V is the name of the diet used for the maintenance of the stock colony and also of all positive control groups. The experimental groups received a diet called Pork I which was synthetic except for the protein furnished in the form of dried, autoclaved pork muscle.

The main plan of the experiment including Series I through Series IV and the distribution of animals in the various experimental groups are summarized in Table 1.

TABLE 1. SUMMARY OF EXPERIMENTAL PLAN

Series	Name of diet fed	Reproductive status	Post-starvation treatment		Analyses performed	No. of rats
			Food given and quantity	Length of period		
I	Steenbock V	Pregnant	4 gm. Steenbock V	7 hrs.	Maternal liver glycogen Fetal liver glycogen	23 10
		Virgin	4 gm. Steenbock V	7 hrs.	Liver glycogen	8
	Pork I	Pregnant	4 gm. Pork I	7 hrs.	Maternal liver glycogen Fetal liver glycogen	26 2
		Virgin	4 gm. Pork I	7 hrs.	Liver glycogen	8
II	Pork I	Pregnant	4 gm. Steenbock V	7 hrs.	Maternal liver glycogen Fetal liver glycogen	10 10
III	Pork I	Pregnant	---	---	Liver glycogen, and reducing substances present in gastrointestinal tract	5
		Virgin	---	---	Liver glycogen, and reducing substances present in gastrointestinal tract	5
	Steenbock V	Pregnant	---	---	Liver glycogen, and reducing substances present in gastrointestinal tract	5
		Virgin	---	---	Liver glycogen, and reducing substances present in gastrointestinal tract	5

TABLE 1. (cont'd.). SUMMARY OF EXPERIMENTAL PLAN

Series	Name of diet fed	Reproductive status	Post-starvation treatment		Analyses performed	No. of rats
			Food given and quantity	Length of period		
III cont'd	Pork I	Pregnant	2⅔ cc. 50% glucose	4 hrs.	Liver glycogen, and reducing substances present in gastrointestinal tract	8
		Virgin	2⅔ cc. 50% glucose	4 hrs.	Liver glycogen, and reducing substances present in gastrointestinal tract	8
	Steenbock V	Pregnant	2½ cc. 50% glucose	4 hrs.	Liver glycogen, and reducing substances present in gastrointestinal tract	8
		Virgin	2⅔ cc. 50% glucose	4 hrs.	Liver glycogen, and reducing substances present in gastrointestinal tract	8
IV	Pork I	Pregnant*	2½ cc. 50% glucose	4 hrs.	Liver glycogen, and reducing substances present in gastrointestinal tract	5

*Killed on the twenty-second day of gestation.

EXPERIMENTAL PROCEDURE

ANIMALS USED

General

The animals used in the study herein described were albino rats (*Mus norvegicus albinus*) of Wistar stock, strain A, belonging to the stock colony of the Foods and Nutrition Department at the Iowa State College. These rats were of known genetic history and for about eighty generations had been inbred by brother and sister matings. The animals composing the experimental groups in this study were taken from the stock colony over a period of four years and represent the nineteenth to the thirty-first generations bred in the Iowa State College laboratory.

The composition of the stock diet was permanently established in 1932. It represented a modification of a whole grain diet originally described by Steenbock in 1923 and was designated as Steenbock V. The quality of the ingredients used in the ration was kept as uniform as possible throughout the years.

Young female rats were taken at the time of weaning

from the second or third litters of the breeding stock. Uniform animals as judged from the weights at birth and at weaning were chosen. Until the time of sexual maturity, determined by the date of the opening of the vaginal orifice, all experimental animals received the stock colony ration, Steenbock V. At the onset of sexual maturity, the diet of the rats placed in the experimental groups was changed to Pork I while the control groups continued to receive Steenbock V. All animals were transferred to individual cages on the day of the opening of the vagina.

In Series III, in so far as possible, the representatives of a litter were distributed evenly among the groups. The virgin controls for Series I were in littermate pairs with each other but not with the pregnant animals, since the studies were made in successive years. The distribution of animals and the number of each rat appear in Table VI and Table VII (Appendix).

The system of brother and sister matings employed in the stock colony was followed in breeding all experimental animals. All males were maintained on the stock colony ration.

Uniformity of Animals Used

The uniformity of the animals used in the experiment may be judged by using as indices: body weight at time of weaning, age at sexual maturity, body weight at sexual maturity, age at initiation of first pregnancy, and body weight at initiation of the first pregnancy. The average values for each of the experimental groups are given in Table 2. The variation is no greater than that which has been demonstrated as characteristic of the stock colony (Greenwood, 1940).

TABLE 2. UNIFORMITY OF ANIMALS USED IN THE EXPERIMENT[1]

Series	Experimental group	Number of animals	Body wt. at time of weaning	Age at sexual maturity	Body wt. at sexual maturity[2]	Age at initiation of first pregnancy	Body wt. at initiation of first pregnancy
			gm.	days	gm.	days	gm.
I	Steenbock V						
	Pregnant	23	52.00	45.13	92.86	68.91	136.95
	Virgin	8	53.75	40.60	90.33	---	---
	Pork I						
	Pregnant	26	51.27	44.21	91.33	71.67	143.25
	Virgin	8	51.75	42.87	92.12	---	---
II	Pork I						
	Pregnant	10	50.50	44.50	91.60	63.00	128.40
	Pork I						
	Pregnant	5	50.40	42.40	88.00	74.80	149.00
	Virgin	5	51.00	43.40	93.40	---	---
III	Steenbock V						
	Pregnant	5	51.00	47.40	92.20	73.20	137.00
	Virgin	5	49.00	46.75	89.66	---	---
	Pork I						
	Pregnant	8	46.37	43.75	82.00	69.62	129.12
	Virgin	8	49.12	44.50	86.50	---	---
	Steenbock V						
	Pregnant	8	52.50	42.75	85.60	68.75	130.00
	Virgin	8	50.87	41.12	83.12	---	---
IV	Pork I						
	Pregnant	5	48.20	40.80	82.80	72.60	148.20

[1]These data include animals studied over a period of four years.
[2]Opening of vaginal orifice.

COMPOSITION AND PREPARATION OF DIETS

Steenbock V

Steenbock V, the ration fed the control group, consisted of a basal and supplementary portion. The formula of the basal portion was as follows:

Yellow cornmeal[1]	64.0 gm.
Crude casein[2]	5.0 "
Linseed meal[3]	16.0 "
Ground alfalfa[4]	2.0 "
Sodium chloride[5]	0.5 "
Calcium carbonate[6]	0.5 "
Yeast[7]	1.5 "
Irradiated yeast[8]	0.5 "
Wheat germ[9]	10.0 "
	100.0 "

1. Purchased from Grain Storage, Iowa State College.
2. Finely ground, purchased from the Wilkens-Anderson Co., Chicago, Illinois.
3. Purchased from Ames Grain and Coal Co.
4. Purchased from Ames Grain and Coal Co.
5. Purchased in local market.
6. Purchased from Chemistry Stores, Iowa State College.
7. Yeast foam tablet powder purchased from Northwestern Yeast Co., Chicago, Illinois.
8. Irradiated in 200 gm. lots for 20 min. at a distance of 15 in. with a quartz mercury lamp.
9. Pure, purchased from Washburn Crosby Co., Minneapolis, Minnesota.

The supplementary portion of the diet consisted of milk, meat, and lettuce prepared and fed as follows: Dried whole milk[1] was purchased in sufficient quantities for one year's feeding. To prepare liquid milk for use, 130 gm. of milk powder was mixed with 1 qt. of distilled water in a Hobart mixer at high speed. One teaspoon of cod liver oil[2] and 2 cc. of a solution[3] containing small amounts of potassium, manganese, aluminum, and copper were added to each quart of dried milk.

The quantities of reconstituted milk fed are described below:

Male	12.5 cc. daily
Resting female	12.5 cc. daily
Pregnant female	25.0 cc. daily
Lactation female	50.0 cc. daily

The lettuce consisted of discarded outside leaves from head lettuce and was obtained from the Memorial Union Cafeteria or the Home Economics Tea Room. Fresh leaves were chosen, washed in cold water, and 10 gm. fed to each rat three times per week.

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1. The powdered milk used was Klim, a product distributed by the Borden Co., New York. The milk purchased represented winter milk from one day's run in the factory.
2. Refined Norwegian vitamin tested cod liver oil, U.S.P., imported by the Pearson-Ferguson Co., Kansas City, Mo.
3. The solution contains 0.08 gm. of potassium iodide, 0.316 gm. manganese sulfate, 0.098 gm. potassium aluminum sulfate, and 0.875 gm. of anhydrous copper sulfate per 100 cc. of distilled water.

Raw beef round, freshly ground, was obtained from a local market and fed as the meat supplement. The meat was measured with an aluminum spoon calibrated to hold 5 gm. and was given three times each week. Meat and lettuce were fed on alternate days.

Pork I

The experimental ration containing pork had the following composition:

Canned pork muscle (dried to one-half its original weight)	25 gm.
Cornstarch[1]	53 "
Yeast[2]	5 "
Agar agar[3]	2 "
NaCl[4]	1 "
Salt mixture[5]	4 "
Butterfat	8 "
Cod liver oil[6]	2 "
	100 "

The quality of the protein in the ration was kept as uniform as possible by purchasing green skinned hams in lots varying in size from 300 to 1000 pounds. The hams

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1. Purchased in the local market in 280 lb. lots.
2. Yeast foam tablet powder purchased from Northwestern Yeast Co., Chicago, Ill.
3. Bacto-Agar purchased from the Difco Laboratories, Inc., Detroit, Mich.
4. Purchased in local market.
5. Osborne, T. B., and Mendel, L. B., J. Biol. Chem., 37, 557-601, 1919.
6. Refined Norwegian vitamin tested cod liver oil, U.S.P., purchased from the Pearson, Ferguson Co., Kansas City, Mo.

were stripped of excess fat, boned, and ground once through the medium plate of a meat grinder. The ground meat was packed in No. 2 enameled tin cans, 1 lb. to a can; the cans sealed and processed in pressure cookers for 65 minutes at 15 pounds pressure. After cooling in cold running water, the cans were examined for leaks and stored at room temperature until needed.

To prepare the pork for use the cans were opened, all visible fat removed, the contents spread out on metal trays covered with cheese cloth and dried in a warm oven at 90-100° C. One thousand gm. of meat was spread on one tray and dried to one-half the original weight, which usually required one to two hours.

The butterfat used was prepared from butter purchased at the Iowa State College Dairy. Four lbs. of butter were heated in a double boiler for two hours. The coagulated protein and the salt which rose to the surface during the heating process were skimmed off, the liquid fat decanted and filtered through a plug of cotton in a funnel with a copper hot water jacket. The fat was cooled and stored in the refrigerator until needed.

Various precautions were taken to prevent the production of rancidity in the rations fed. The pork diet was prepared twice weekly by mixing in a Hobart

mixer and stored in the refrigerator in closed, enameled tin cans. Fresh diet was given the rats each day and the uneaten portions discarded.

The pork diet described, which was synthetic except for the source of protein, was supposedly adequate for the nutrition of the rat. Armstrong (1939) has discussed the experimental evidence in support of this statement.

CARE OF ANIMALS

After reaching sexual maturity the animals were kept in individual round wire mesh cages. These cages were arranged on steel tiers and were placed in shallow enamel pans lined with paper towels. Coprophagy was prevented to a large extent by the raised wire mesh bottom of the cage, since feces and urine dropped through to the absorbent toweling beneath.

Food and water were given ad libitum. The food was offered in glass jars attached to the side of the cage by means of copper wire. Distilled water was provided from glass bubble fountains inserted through openings in the cages. The animals maintained on the Steenbock ration received milk daily in small porcelain cups held in metal brackets. All dishes were changed three times a week, washed in strong soap suds, and sterilized in live

steam for 15 minutes. The cages, pans, water fountains, and metal holders were washed and sterilized weekly.

The routine which was followed daily except for Sunday, consisted of changing the paper towels under the cages, observing the general well-being of the animals, filling water fountains if necessary, and providing food. The food consumed by each rat receiving Pork I was carefully weighed, enough food being given each day so that a surplus of from 2 to 5 gm. remained. This uneaten portion was discarded on the following day. A double portion of food was given on Saturday. The quantity of food consumed by the rats fed the Steenbock diet was not recorded, but an ample supply was always provided.

Each animal was weighed weekly and pregnant females were weighed daily for the week preceding and the week following parturition. In addition, the pregnant females were weighed on the twenty-first day of gestation at 10 p.m., on the twenty-second at 8 a.m., 12 m., 4 p.m., 8 p.m., and 10 p.m., or until parturition occurred. This frequent handling of the animal permitted the early observation of toxic symptoms, as well as the collection of data concerning the number, weight, and condition of the young born.

As soon as possible after parturition the young were

counted and weighed. The live babies were replaced with the mother and the cage provided with nesting paper. The lungs of the young found dead were removed and placed in water. If the tissue floated it was assumed that the individual had drawn air into its lungs and was therefore born alive. On the fourth day the litters were reduced to six in order to equalize the strain of lactation on the mother. If the size of the litter permitted, three males and three females were retained. Those young were chosen whose weights most closely approached the average individual weight for the litter. If less than six rats had been born the mother was permitted to rear the whole litter. The litter as a whole was weighed daily and the males and females were weighed separately on the fourth, seventh, fourteenth, twenty-first, and twenty-eighth days. The litter was weaned when 28 days old and the individual weights recorded.

The temperature of the room in which the rats were housed was kept as uniform as possible. An examination of the daily records of the temperature showed a variation of from 75 to 80 degrees Fahrenheit. An attempt was made to keep the air comparatively free from dust by placing a cloth filter over the door into the hall and by the use of an oily compound in sweeping the floor. The

rats were removed from the room once a month and the room sprayed with an insecticide.

VAGINAL SMEAR TECHNIQUE

The animals were mated according to the vaginal smear technique. The five stages that make up the rhythmical changes occurring during the oestrous cycle of the female rat may be followed by a daily histological study of the cells of the vaginal epithelium (Long and Evans, 1922). In order to obtain these cells for examination, the rat was held on her back in the left hand with her head supported by the thumb and index finger. The tail was held by the fifth finger of the right hand, and a small glass rod (2 mm. in diameter), the ends carefully fire polished, gently inserted into the vagina. The rod was withdrawn and touched to a drop of distilled water on a microscope slide. The cells from the vaginal mucosa, which adhered to the rod and were washed into the water, could be identified easily by examination under low power of a microscope using artificial illumination.

To avoid the possibility of spreading vaginal infection the rods used were immediately placed in a strong soap solution. Later they were washed with a stiff brush, inserted in test tubes containing about

5 cc. of water, the tubes plugged loosely with cotton, and sterilized for 20 minutes at 15 pounds pressure in an autoclave.

The stages of the oestrous cycle were identified by the kind of cells predominant in the smear according to the classification of Long and Evans (1922). This classification is shown below:

Stage 1. Epithelial cells, the pro-oestrous period

Stage 2. Epithelial and cornified cells, the oestrous period

Stage 3. Many cornified cells

Stage 4. Cornified cells and many leucocytes, the metoestrous period

Stage 5. Leucocytes, epithelial and cornified cells, the dioestrous period

Vaginal smears were examined daily at approximately the same hour, and a record kept of the date, time of day, weight of rat, stage of the oestrous cycle, and any observation concerning the physical appearance of the female. After the birth of a litter the young were weighed daily and their condition noted.

The female rat will normally accept copulation only toward the end of stage one and in the early part of stage two. The study of vaginal smears was initiated when the rat reached the age of eight weeks and at the

second appearance of stage one or stage two, a brother male was introduced into the cage. The next day the female was examined for positive signs of mating, judged by the presence of a vaginal plug in the vagina or under the cage, or by the presence of sperm in the smear. If mating had occurred the male was removed but if no signs of mating were evident the male was left in the cage until the appearance of stage three in the smear. If the female refused to accept the male for three consecutive cycles, another male of proved fertility was used.

If the mating was positive, the smear was carefully examined from the twelfth to the seventeenth days for the implantation sign as indicated by the presence of red blood cells in the vagina.

Vaginal smears were not taken on the virgin rats studied, but all other rats were observed daily from the ninth week until killed.

TREATMENT OF RATS BEFORE KILLING

Starvation

All pregnant animals except those of Series IV and Series V were killed on the 21.5 day of the second pregnancy. These rats had thus all undergone the strain

of one pregnancy and had been given the opportunity to rear one litter. As soon as possible after the first litter was weaned, or after the death of the first litter, the mother was mated again. Thus, pregnant females with similar previous histories, and approaching parturition, were studied.

It was necessary to starve all animals to deplete the reserves of liver glycogen to a normal level before the experimental feeding. Cori (1926) starved his rats for 48 hours and found that after starvation the average liver glycogen content of seven males was 0.397 gm. per 100 gm. of liver. Other workers, for example, Gregg (1931) and Wilson and Lewis (1930), have reported using a 24-hour starvation period. Since many of the animals investigated in the study here reported were pregnant and approaching parturition, it was thought that even a 24-hour starvation period would impose too severe a physical strain upon the animal. Consequently a 13-hour period was chosen and the amount of glycogen determined in livers removed from pregnant and virgin females fed the Steenbock and Pork I diets. The results are shown in Table 3. Except for one exceptionally high value none is as high as Cori's average, probably because muscle glycogen has not as yet been mobilized. Goldschmidt,

Vers, and Revdin (1939), on the other hand, report values for liver glycogen after 24 hours of starvation that correspond closely to those obtained in this investigation, i.e., 0.12 and 0.10 per cent. The experimental procedure used herein undoubtedly (Table 3) reduces preformed glycogen to a uniform level.

The food was removed from the cage at 6 p.m. on the twentieth day of gestation and the experimental feeding begun at 7 a.m. on the following morning. In view of the late appearance of toxic symptoms it might have been more desirable to postpone the starving and feeding for 24 hours, but since many litters are born before noon on the twenty-second day, it was thought that the occurrence of parturition before the tissues could be removed for analysis would necessitate the loss of too many animals.

The virgins were killed when they were approximately the same age as the pregnant females.

All animals were weighed before and after starvation and the weights recorded.

Method of Feeding

Solid Food

After the 13-hour starvation period, all animals in Series I were given 4 gm. of the diet upon which they had

TABLE 3. GLYCOGEN CONTENT OF LIVERS OF FEMALE RATS FASTED PREVIOUSLY FOR 13 HOURS EXPRESSED AS GM. PER 100 GM. WET WEIGHT OF LIVER

Pork I		Steenbock V	
Virgin	Pregnant	Virgin	Pregnant
.0830	.0360	.4089	.0477
.1327	.0463	.0894	.0435
.2449	.0347	.3013	.0495
.1178	.0322	.0879	.0488
1.8296	.0358	.0632	.0404
Av. .1446[1]	.0370	.1901	.0460

1. This average does not include the extremely high value. When included the average is .4816.

been maintained. This amount was weighed on a trip balance into a small porcelain supplement dish and placed in the cage. At the end of four hours any food remaining uneaten was removed and weighed. The weight of food to be offered to the starved animal was set at 4 gm. because it was desirable to provide as large an amount as most animals would be able to consume. The average weight of the food eaten and the per cent of animals that ate the entire amount are shown in Table 4. Without exception, the virgin rats consumed all the food provided and the pregnant rats reared on the Steenbock ration consumed an average of 3.9 gm. The fact that the pork-fed rats on the whole ate less, may have been due to the fact that they were generally in less vigorous physical condition than those reared on the stock diet.

Three hours after the removal of the food cup, or seven hours after the initiation of feeding the rats were killed. Cori (1926) has stated that the maximum glycogen retention in the liver occurs four hours after the feeding of glucose solutions by means of a stomach tube. Since the digestion and absorption time is much shorter for glucose than for a solid ration such as Steenbock V or Pork I, and since the solid food was consumed intermittently over a period of several hours,

TABLE 4. POST-STARVATION FOOD CONSUMPTION OF RATS FROM SERIES I AND SERIES II

	Series I				Series II
	Steenbock V		Pork I		Pork I
	Pregnant	Virgin	Pregnant	Virgin	Pregnant
Number of animals	23	8	26	8	10
Av. wt. eaten in gm.	3.9	4.0	3.7	4.0	3.7
Per cent of animals who ate 4 gm.	86.96	100.0	76.92	100.0	70.00

seven hours after the offering of food was more or less arbitrarily chosen as the time of analysis.

The pork-fed animals of Series II were treated similarly to those of Series I except that 4 gm. of the Steenbock ration instead of Pork I were given after the starvation period. The exact duplication of the experimental procedure in the two groups made possible a direct comparison of the glycogen content of the livers of these animals with that of the animals fed Pork I after starvation for the detection of possible differences caused by the quality of the post-starvation diet.

Glucose Solutions

To avoid variations in both the quality and quantity of the food given following the starvation period, groups of pregnant and virgin rats of Series III reared on both the Steenbock V and Pork I diets were administered a known amount of glucose solution by means of a stomach tube. The amount of intestinal absorption as well as the quantity of liver glycogen was determined. The animals were starved for 13 hours in the same manner as those of Series I and Series II; that is, the food was removed at 6 p.m. on the twentieth day of gestation and feeding instituted at 7 a.m. on the following day.

The dextrose[1] used in making the solutions was of high quality and a sufficient quantity was purchased for the entire experiment. A 50 per cent solution was made by dissolving 12.5 gm. of glucose in warm distilled water in a 25 cc. volumetric flask, cooling, diluting to the mark, and mixing. The solution was made up about 18 hours before it was used and was stored in the refrigerator to prevent changes due to fermentation. A fresh solution was made each time glucose was administered.

In general, Cori (1925) used 2.5 cc. of 50 per cent sugar solutions for comparative studies because enough sugar could be introduced to allow absorption to proceed for at least three hours. More concentrated solutions were avoided because of their greater viscosity. He observed also that if more than 2.5 cc. were given, diarrhea appeared.

The method used for the administration of the glucose solutions was essentially that described by Cori (1925). A urethral catheter No. 8 was used for a stomach tube and softened before use by plunging for a moment into a beaker of boiling water. A 5 cc. Record type of syringe which had a metal plunger was used to measure the solution fed.

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1. Baker's Analyzed, purchased from Arthur H. Thomas, Philadelphia, Pennsylvania.

In the morning, prior to the feeding of the starved rat, the glucose solution was removed from the refrigerator and warmed in a stream of hot water. The syringe was filled with the warm glucose solution, air bubbles expelled, and the level of the solution carefully adjusted to the 2.5 cc. mark. The catheter was firmly attached over the hypodermic needle to the neck of the syringe. In view of the volume of solution remaining in the catheter after feeding, as shown by the analysis of the solution carried on simultaneously, more glucose could have been introduced into the stomach of the rat by using a larger initial volume in the syringe. If allowance is made for the volume of solution remaining in the rubber tube, a volume of 3.0 cc. as measured in the syringe would probably deliver about 2.5 cc. into the rat.

A small wooden block with a hole in the center, was placed in the mouth of the rat to facilitate the introduction of the tube. The size of this block was such that it could be slipped between the jaws easily without causing pain to the animal. The rat was held in a ventral position in the left hand of one technician who restrained the front legs with her thumb and fore finger. She also, with her right hand, held the block between the teeth of the rat. A second operator then slipped the end of the catheter through the hole in the mouthpiece and

gently introduced the tube into the rat's stomach. A mark on the tube was found useful as an indication of the depth to which it had to be inserted. As soon as the tube was in place, the sugar solution was slowly expelled from the syringe into the stomach. The entire procedure required only a few minutes and generally no difficulty was encountered. The rats seemed to experience little discomfort.

After the solution was delivered, the catheter with the syringe still attached, was quickly withdrawn from the oesophagus and the rat replaced in her cage.

The quantity of glucose introduced into the stomach was determined for each rat. Cori (1925) estimated this amount by delivering into a volumetric flask the same amount of sugar solution under exactly the same conditions as when the rats were fed. Although not specifically stated by Cori, apparently the residual contents of the catheter were discarded each time. This method was used for a few rats at the beginning of the study here reported, then a modification was practiced.

The modified method was suggested by Pierce[1] and may be described as follows: As soon as the catheter was removed from the oesophagus of the rat it was quickly

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1. Personal communication from H. B. Pierce.

placed in the neck of a 1000 cc. volumetric flask. The syringe was then removed from the catheter and the contents of the catheter rinsed into the flask by repeated washings with distilled water from a narrow stream delivered by a wash bottle. The tube was removed from the flask, the solution diluted to the mark, mixed, and aliquots taken for glucose analysis. The syringe was again filled with the glucose solution to the 2.5 cc. mark, the catheter attached, and the solution introduced into a second 1000 cc. volumetric flask. This time the catheter was held in the flask after the syringe was removed and the tube washed into the flask in the manner previously described. The solution was diluted to the mark, mixed, and aliquots removed for analysis. Thus, the first flask contained the washings from the catheter and the second contained the washings plus the equivalent of the solution received by the rat. The difference should represent the amount of glucose administered to the rat.

Each method of glucose administration was tested. Two rats previously fasted for 13 hours were fed 2.5 cc. of a 50 per cent glucose solution in the manner described for each procedure. They were killed immediately and the gastrointestinal contents analyzed. The quantity of reducing substance recovered in each test was compared

with the quantity of glucose administered as calculated from the analyses of the solutions. The results were as follows:

TABLE 5. RECOVERY OF GLUCOSE FROM INTESTINAL TRACT

Method	Glucose administered	Glucose recovered	Per cent recovered
	mg.	mg.	
Cori			
Rat no. 1	859.64	834.89	97.12
Rat no. 2	857.66	827.90	96.53
Pierce			
Rat no. 1	855.36	858.98	100.42
Rat no. 2	879.68	866.66	98.52

Description of Animals

The general physical condition of each animal was observed just before it was killed. A record was kept according to the outline shown in Form I in the appendix.

REMOVAL OF TISSUES FOR ANALYSIS

Method of Killing the Animal

Certain physiological factors are known to influence the glycogen content of the liver by producing marked hyperglycemia. It was obviously desirable to control these factors in so far as possible in the period preceding the death of the animal. The method used to kill

the animal may also affect liver glycogen. Peters and Van Slyke[1] list the common physiological conditions which deplete liver glycogen as starvation, exercise, and cold. The control of the starvation factor has already been discussed. Since the rats were starved during the night, fed in the morning, and killed about noon, they usually slept during the intervening hours, so that the effect of exercise was probably negligible. The animals were kept at the temperature to which they were accustomed which rules out that variable.

Various considerations were taken into account in choosing the method for killing the animals. The effect of anesthetics upon blood sugar levels and consequently upon liver glycogen is well known. Stander and Radelet (1926) found that ether, chloroform, and nitrous oxide produced marked hyperglycemia in dogs. Ravdin, Vars, Goldschmidt, and Klingensmith (1938) observed that within a few minutes following administration of an anesthetic a hyperglycemia occurs which nearly always increases during the period of anesthesia and results in a loss of liver glycogen. Epstein and Aschner (1916), working with human subjects, concluded that anesthesia plays an important role in the production of hyperglycemia after surgical

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1. Peters, J. P., and Van Slyke, D. D. 1931. Quantitative clinical chemistry, Vol. I. Interpretations, Williams and Wilkins Co., Baltimore.

procedure. The action of drugs may be hypoglycemic or hyperglycemic depending upon the effect on the nervous system (Peters and Van Slyke[1]). The use of anesthetics was therefore avoided.

Some workers, for example, Hrubetz and Dotti (1934) and Marble, Grafflin and Smith (1940) have decapitated the animals. However, loss of blood is known to produce hyperglycemia which according to Epstein and Baehr (1914) is a compensatory response on the part of the organism to keep the total blood sugar up to a level commensurate with the needs of the tissues. The effect of loss of blood upon liver glycogen was brought out in the course of the experiment here reported by rat No. 26270 in Series III. During the process of feeding, a toe nail was injured and some bleeding occurred during the four-hour absorption period. Upon analysis the liver was found to be almost depleted of glycogen.

It was finally decided to stun the animals by a blow at the base of the brain, then remove the tissues for analysis. This method was used by Cori and Cori (1926) and by Gregg (1931) and has been in use in the Nutrition Laboratory at Iowa State College for some years. It was

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1. Peters, J. P., and Van Slyke, D. D. 1931. Quantitative clinical chemistry. Vol. I. Interpretations, Williams and Wilkins Co., Baltimore, p. 112.

an additional advantage to use the same method which had been practiced in other studies from this laboratory and a method to which the workers were accustomed.

Removal of Liver

Extirpation of Maternal Liver

After the animal was stunned it was firmly held in a ventral position and an incision made on the ventral median line extending from the anus to the diaphragm. The abdominal wall was cut transversely from the median incision, thus exposing the viscera. Care was taken to avoid cutting the larger blood vessels.

The liver was removed as quickly as possible by severing the mesenteric attachments and the blood vessels. Since glycogen is subject to rapid post mortem changes it was essential to work fast. The excised liver was blotted free of blood and any adhering fat or connective tissue trimmed off. It was then divided approximately into halves, each half cut into strips and dropped into tared tubes of potassium hydroxide. During these operations the color and consistency of the organ were noted.

As soon as the liver was removed the medial abdominal incision was extended anteriorly through the diaphragm and the ribs to expose the heart and lungs to kill the

animal. In most cases the heart was found still beating.

Extirpation of Fetal Liver

The uterus of the pregnant animals was ligated at the oviducts and the cervix, and removed by cutting posterior to the cervical ligature. Some fat and the ovaries remained attached to the uterus when it was removed. The intact organ was weighed roughly on a trip balance and the weight recorded. It was then split longitudinally and the number and condition of the feti and the number of resorptions, if any, observed. The feti, together with the placentae, were freed from the uterine wall and the stripped uterus weighed on the trip balance. The corpora lutea were examined and counted.

In cases where the fetal livers were to be analyzed, the feti were anesthetized by injecting a very small amount of nembutal (about 0.1 cc.) into the pleural cavity. The liver was then exposed by making a medial abdominal incision and two transverse incisions, and it was removed with a pair of small forceps. The livers from all the feti of one female were dropped into one tared tube of potassium hydroxide. Some difficulty was encountered in removing the entire liver due to the extreme friability and small size of the fetal organ. How-

ever, the liver was very conspicuous due to its color and size in relation to that of the fetus.

Removal of Gastrointestinal Tract

The gastrointestinal tract was removed for examination from all animals composing Series III and Series IV. In general, the method of removal was that described by Cori (1925) and later by Wilson and Lewis in 1929. Ligatures were placed around the oesophagus and the rectum, and the stomach, small intestine, and whole large intestine were carefully detached from the mesentery and placed on a paper towel. At this point originally, the tract was ligated posterior and anterior to the cecum and the cecum only discarded. Later, following the advice of Pierce[1], the ligature was placed anterior to the cecum and the large intestine discarded as well. This procedure avoided contamination of the subsequent washings with large amounts of fecal material. The method probably introduced no appreciable error because it is believed that absorption of sugar is completed anterior to the region disregarded.

Excess adhering fat and portions of the mesentery were trimmed off and the whole intestinal tract opened

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1. Personal communication from H. B. Pierce.

longitudinally and dropped into a beaker. This process was found to be quite simple if the tract were stretched on a paper towel, one end picked up with the forceps in the left hand, held over the beaker, and the point of a pair of small scissors inserted into the open end. The organ was slit rapidly by holding the scissors nearly stationary and pulling the split portion into the beaker with the forceps. In this way none of the contents of the intestine were lost.

The instruments were rinsed into the beaker with hot distilled water and the tract rinsed repeatedly with portions of water. The washings were transferred quantitatively into a 250 cc. volumetric flask and set aside for analysis.

Cori (1925) stated that there is little loss of sugar due to bacterial action in the excised intestine during a period of three to five hours at 37° C. On this basis it was thought that procedures in the present study might be facilitated if the tract could be frozen and preserved for analysis on the following day. To test this procedure a starved rat was fed 2.5 cc. of a 50 per cent glucose solution in the manner previously described, stunned immediately, and the gastrointestinal tract removed and placed in a beaker of carbon dioxide snow. It

froze immediately. The beaker was then packed in dry ice and set away in the refrigerator. Twenty-four hours later it was thawed at room temperature, split open, washed, and the contents analyzed. Fermentation had obviously taken place since the titration value was considerably reduced (15.40 cc. vs. an average of about 19.00 cc. observed on tracts analyzed immediately). Therefore, the plan was abandoned and all intestinal tracts were washed immediately after removal and the solutions analyzed within a few hours.

Examination of Tissues

After the liver, uterus, and gastrointestinal tract had been removed and treated as described previously, observations were made of the internal condition of the animal and recorded as shown in Form II (Appendix).

The relative amounts of fat in the subcutaneous, peritoneal, omental, perirenal, genital, and intermuscular depots were noted. The liver was examined at the time of its removal and the condition recorded after it had been placed in the potassium hydroxide. The kidneys were detached by cutting the renal blood vessels, and split longitudinally. The color and consistency of the cortex, medulla, and pelvis were then noted. The pancreas

had been observed at the time of removal of the gastro-intestinal tract. The condition of the reproductive organs of the pregnant females, number of feti, number of corpora lutea, and number of resorptions were recorded when the uterus was removed. The stomach was split open, washed in running water, and examined for ulcers. The lungs were removed and studied for signs of infection, and the base of the tongue and middle ear exposed for the detection of pus pockets. Any other abnormal conditions were recorded.

CHEMICAL METHODS

Treatment of Liver

Treatment of Maternal Liver

Glycogen was determined essentially as described by Good, Kramer, and Somogyi (1933). This method is a modification and simplification of the classic Pflüger (1904) method which makes it possible to perform the analysis in a few hours. The process was carried on in 50 cc. Pyrex centrifuge tubes with round bottoms and pourout spouts. As stated by Good *et. al.* (1933), the round bottomed tubes make it possible to mix the contents by agitation while the use of pointed centrifuge tubes

requires mixing with a stirring rod. These tubes were pierced by two small openings on opposite sides near the upper edge and fitted with a bail made of fine non-corrosive piano wire, so that they could be hung from the beam of an anlytical balance. They were permanently labeled by scratching numbers near the top with a carbide pencil.

On the day that an animal was to be killed, two tubes were charged with 8 cc. of 30 per cent potassium hydroxide. This amount allows for approximately 2 cc. per gm. of tissue, the proportion recommended by Good, Kramer, and Somogyi. The potassium hydroxide was prepared according to directions given in the appendix, and each year's supply stored in a reagent bottle provided with an automatic burette and fitted with soda lime tubes. After the introduction of the liver, the centrifuge tubes were tightly stoppered with corks covered with tin foil, weighed on an analytical balance, and set aside until needed. The liver was removed, divided, and submerged in the potassium hydroxide as previously described. The tubes containing the liver were then quickly weighed and immersed in a boiling water bath. Usually about 10 or 15 minutes elapsed between the time of stunning the rat and placing the second tube in the

hot water. However, only about three minutes were required to open the animal, remove the liver and drop it into the potassium hydroxide. Wilson and Lewis (1930) found that no noticeable destruction of glycogen occurred in the time elapsing between the slaughter of their animals and the heating with alkali.

The tubes were kept in a boiling water bath one-half hour or until a homogeneous solution was formed. It was found that occasional gentle agitation facilitated the solution of the liver and prevented small bits of tissue from drying to the sides of the glass. The tubes were removed from the water bath, cooled in a stream of water and the glycogen precipitated by the addition of 9.6 cc. of 95 per cent ethyl alcohol; i.e., 1.2 volumes in relation to the original volume of potassium hydroxide. The tubes were carefully shaken to mix the alcohol and the alkaline liver solution and then replaced in the boiling water bath until the mixture began to boil. During this heating the precipitate became more flocculant and began to settle out. Cori and Cori (1933) found that heating after precipitation makes the glycogen stick to the glass more readily after centrifugation. During the heating care had to be taken lest the mixture froth and boil over. The frothing seemed to occur with the extremely

yellow livers and may have been due to the formation of soaps with the liver fat. The tubes were removed, cooled to room temperature and centrifuged for 10 minutes with the rheostat set at 14. The centrifuging caused the glycogen to adhere to the bottom of the tubes, so that the mother liquor could be easily decanted. Occasionally when only small amounts of glycogen were present the precipitate was not completely thrown down by centrifugation. In such cases, the mixture was shaken up and recentrifuged for a longer period. After decantation and draining, the excess alcohol was removed by heating for a few minutes on the water bath.

Cori and Cori (1933) advocated redissolving the precipitated glycogen in water and reprecipitating it. Consequently 4 cc. of hot water were added to the precipitate and stirred with a fine glass rod. The glycogen dissolved and was then reprecipitated by the addition of 9.6 cc. of 95 per cent alcohol. The alcohol was added from a pipette in such a manner as to wash off the stirring rod and the sides of the tube. The mixture was again heated to boiling, cooled to room temperature and recentrifuged. The precipitated glycogen was now fairly white in color and the supernatant liquid, a clear amber fluid, was poured off. The excess alcohol was again re-

moved by heating and 10 cc. of normal sulfuric acid added. The tubes were loosely covered with tin foil, replaced in the hot water bath and hydrolyzed for three hours.

Sahyun and Alsberg (1931) concluded that sulfuric acid could safely be substituted for the hydrochloric acid used by Pflüger for the hydrolysis of glycogen. Data presented by these workers indicate that while 90 minutes were sufficient for the hydrolysis of the glycogen, no appreciable destruction of sugar took place beyond that time. Consequently, the procedure of Cori and Cori (1933) was followed in the present investigation and a three hour hydrolysis period used as in the original Pflüger method.

After hydrolysis the tubes were tightly stoppered and stored at a low temperature in the refrigerator until glucose determinations could be made.

Treatment of Fetal Liver

The fetal livers were treated similarly. The livers from one litter were pooled, as was previously mentioned, and due to the comparatively smaller mass of tissue to be dissolved, only 5 cc. of potassium hydroxide was used. The glycogen was then precipitated with 6.0 cc. of 95 per

cent alcohol, dissolved in 3 cc. of water, reprecipitated with 6.0 cc. of alcohol and hydrolyzed with 5 cc. of normal sulfuric acid for three hours.

Treatment of Gastrointestinal Contents

The contents of the gastrointestinal tract were washed into a 250 cc. volumetric flask as has been described. While still warm from the hot water used in washing, 5 cc. of 3 per cent acetic acid, 5 cc. of 10 per cent sodium tungstate, and 5 cc. of 0.66 normal sulfuric acid were added to precipitate the proteins. Miller and Lewis (1932) used like amounts of sodium tungstate and sulfuric acid and Wilson and Lewis (1929) stated that 5 cc. of 3 per cent acetic acid provided an optimum pH for the precipitation of proteins. The contents of the flask were cooled, diluted to the mark, mixed and filtered through filter paper with a suction filter. Aliquots were taken for analysis.

Glucose Analysis

Details of Technique

When the tubes of hydrolyzed glycogen were taken for analysis the contents, first, were transferred quantitatively with small amounts of warm distilled water to

50 cc. volumetric flasks. A few drops of phenol red were added as an indicator and the acid contents neutralized by slowly adding approximately normal sodium hydroxide from a pipette. After the color of the solution turned red, a drop or two of normal sulfuric acid was added until the yellow color returned. Good, Kramer, and Somogyi (1933) added base until the hydrolysate was neutralized. However, since the copper reagent to be added was alkaline, Shaffer and Somogyi (1933) recommended that the solutions be made slightly acid.

The contents of the flasks were diluted to the mark and mixed. Dilutions were made and aliquots taken for analysis according to the amount of glucose present. For example, the solutions from the starved rats contained much less glucose than those from the animals of Series I and Series II.

The method described by Shaffer and Somogyi (1933) was used for the glucose determinations. The stock solutions were prepared according to directions given in the appendix.

With an accurate pipette (B.S.), 5 cc. of the sugar solution was measured into a Pyrex test tube (25 x 200 mm.), followed by 5 cc. of the copper reagent added in a manner to rinse the sugar solutions from the walls of

the test tube. Each day an anlysis was made, blanks were also run by measuring two 5 cc. portions of water instead of sugar solutions and adding 5 cc. of the copper reagent. The solutions were mixed by gentle shaking and the tubes covered by sealed glass bulbs. The tubes were placed in a metal test tube rack holding 16 tubes. The rack was then hung in a vigorously boiling water bath for exactly 15 minutes, an interval timer being used. At the end of this period the rack and tubes were removed to a pan of cold water.

When cooled to slightly above room temperature, 2 cc. of a solution containing 2.5 per cent of potassium iodide and potassium oxalate was added. Next, 5 cc. of normal sulfuric acid was added, the bulbs replaced and the solutions mixed well to dissolve the cuprous iodide which precipitated. After standing (covered) 5 to 10 minutes, with occasional agitation, the bulb and walls of the tubes were rinsed with water and the solution titrated with .005 normal sodium thiosulfate. When the brown of the iodine liberated faded to a straw color, 1 cc. of starch indicator was added. The end point was reached when the dark blue of the starch solution changed sharply to the green blue of copper sulfate. For use during titration, stirring rods were made by coiling a piece of fine glass rodding at one end.

The titration value was subtracted from the heated blank titration and the titration difference corrected by a factor if the thiosulfate was not exactly .005 normal. The preparation and standardization of the thiosulfate are given in the appendix. The amount of glucose per 5 cc. aliquot of sugar solution was calculated from a regression based on analyses of pure glucose solutions of known concentration.

Calculation of Glucose

The first copper reagent was prepared in 1938. It was standardized against solutions containing known amounts of glucose and a regression calculated in the following manner.

Seventeen glucose solutions were prepared containing quantities of glucose varying from 0.1596 gm. per l. to 0.3016 gm. per l. Five cc. aliquots of each of these solutions were analyzed according to the method previously described. Analyses were run in duplicate or triplicate and some of the solutions were analyzed on successive occasions. The composition of the glucose solutions and the volumes of 0.005 normal sodium thiosulfate equivalent to each are shown in Table VIII (Appendix). The volumes of sodium thiosulfate were plotted against the mg. of

glucose present in each aliquot. It was observed that the relationship was linear. Consequently a linear regression was calculated, using the statistical method described by Snedecor.[1] The relationship is expressed by the equation: $\hat{Y} = 10.0808\ X - 1.3059$. In the calculations the symbol X was used to represent mg. of glucose per aliquot analyzed and the symbol Y to represent cc. of .005 normal sodium thiosulfate. The symbol $\hat{Y}$ of the equation represents the estimated value of the volume of sodium thiosulfate, or the predicted value of Y.

The two means, expressed as $\bar{X}$ and $\bar{y}$ were 1.1315 and 10.10, respectively. The regression line was drawn by locating three points by calculating the values for $\hat{Y}$ when X equals 0.7980, 1.4130, and 1.0085. The line was found to pass through the point located by the two means $(\bar{x}, \bar{y})$. The various points as plotted, the regression line and regression equation are shown in Figure 2.

Copper reagents prepared in 1940 and 1941 were standardized by analyses with four new glucose solutions. The composition of these glucose solutions and the average equivalent volumes of the .005 normal sodium thiosulfate equivalent from duplicate titrations of each

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1. Snedecor, G. W.
1938. Statistical methods. Collegiate Press, Inc., Ames, Iowa. Revised ed. p. 114-115.

with the two copper reagents are shown in Table IX (Appendix). These eight points were plotted on the graph drawn previously for the first copper reagent and were found to lie upon the regression line (Figure 3). The regression equation, E = 10.0808X - 1.3059, was used, therefore, for the calculations of all unknown glucose values, the volume of the .005 normal sodium thiosulfate being substituted for E.

Calculation of Glycogen

The weight of glucose, as calculated, was converted to glycogen by the use of the factor 0.927 (Pflüger, 1904).

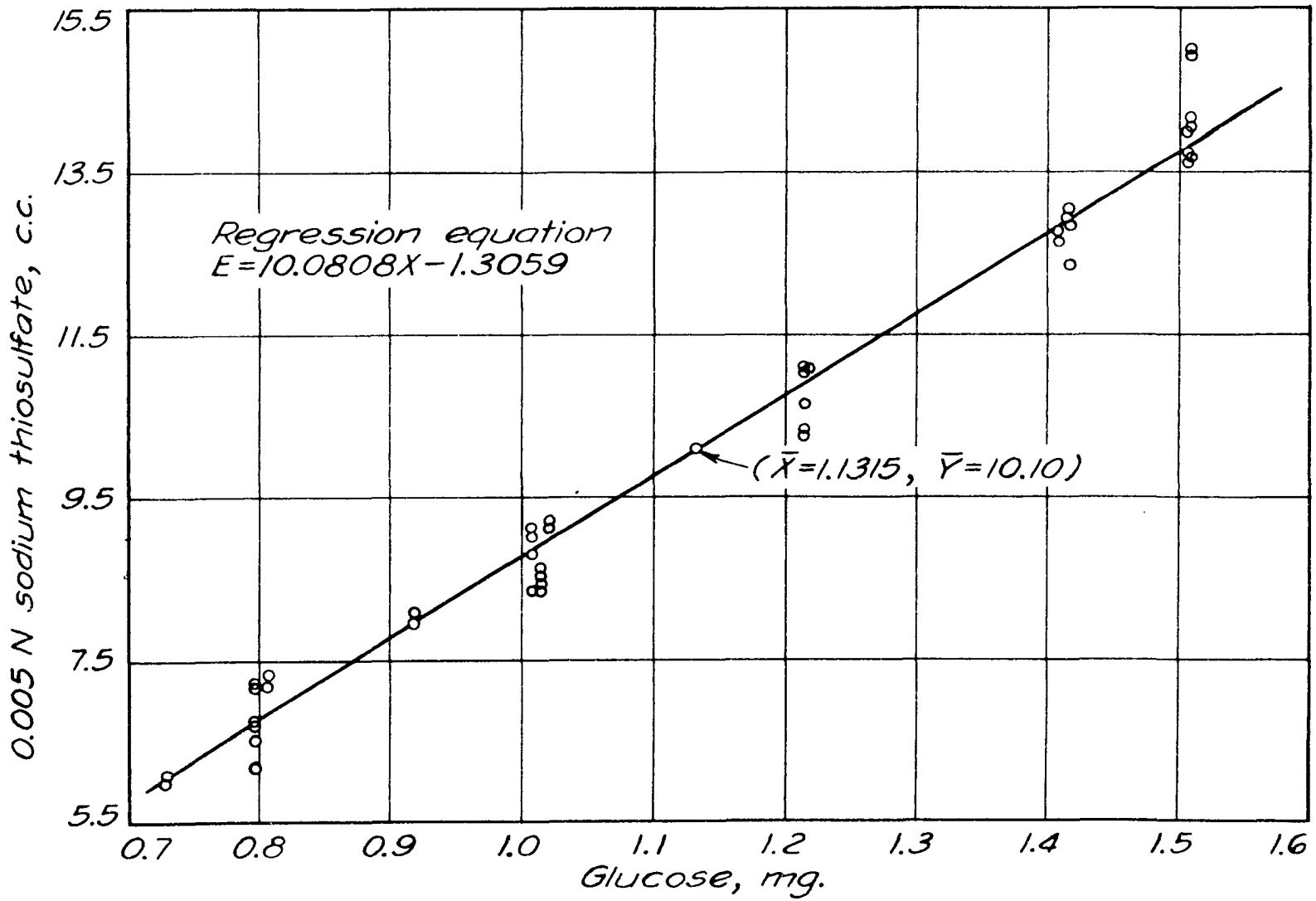

Fig. 2 Regression of number of c.c. of 0.005 normal Sodium Thiosulfate on mg. of glucose calculated for copper reagent 1.

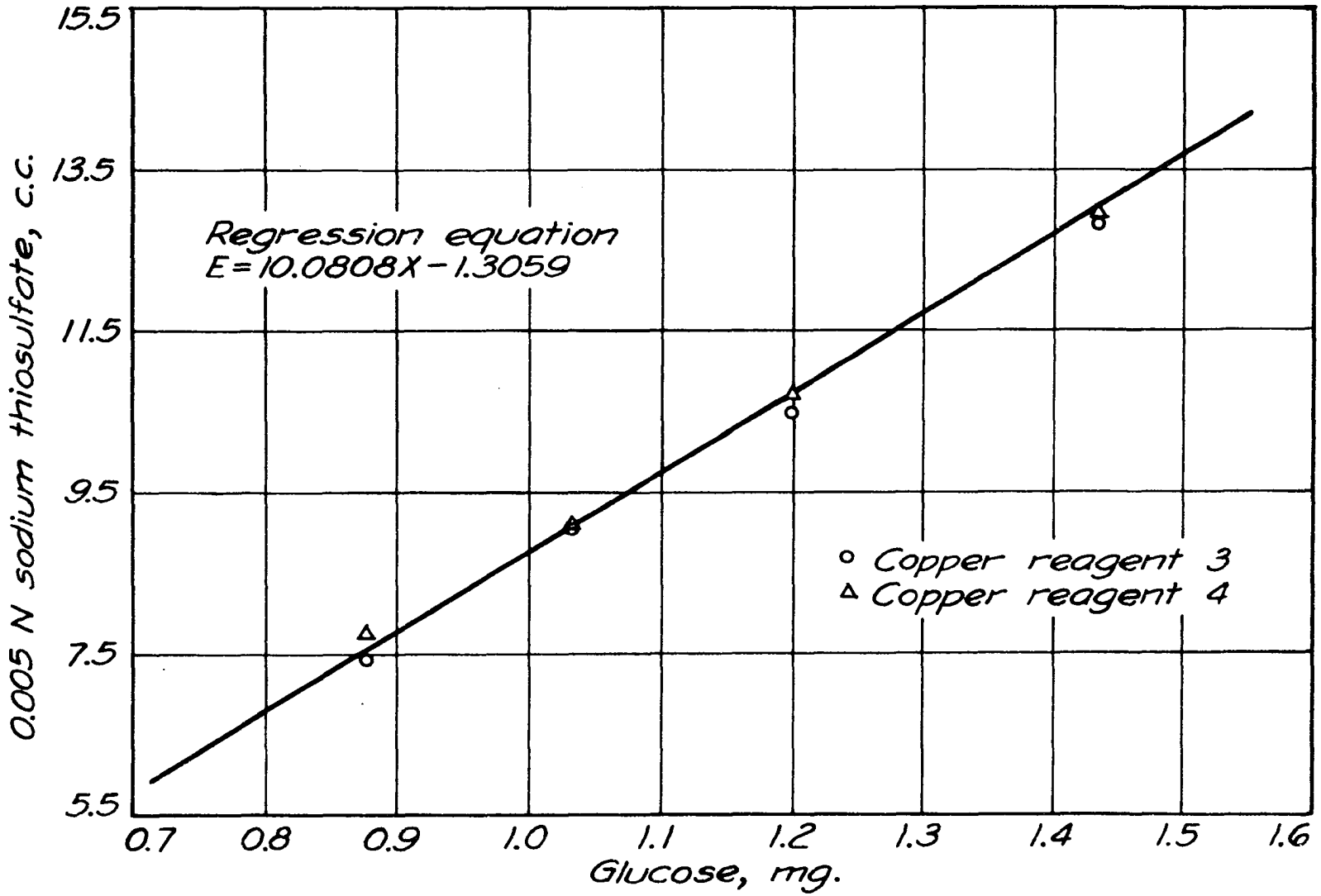

Fig. 3 Standardization of copper reagents 3 and 4.

REVIEW OF LITERATURE

Someone has called the liver the "watchdog of metabolism" and a study of the multiple hepatic functions makes the aptness of the phrase evident. The importance of the organ and its capacity to carry on many metabolic reactions are well known. In fact, because of its versatility, some processes, whose sites are unknown, are ascribed to the liver without experimental evidence.

It is generally known that the liver takes part in the following activities:

1. Secretion of bile
2. Erythropoiesis in the embryo
3. Destruction of erythrocytes
4. Detoxication of poisonous substances brought to it by the blood
5. Removal of particulate substances from the blood
6. Deamination of amino acids and formation of urea
7. Destruction of uric acid
8. The desaturation of fatty acids subsequent to their utilization by the tissues
9. Formation and storage of glycogen and

regulation of the glucose content of the systemic circulation

Other miscellaneous functions are attributed to the liver, such as the production of substances which prevent and cure anemia, the formation of blood proteins, the formation of heparin, the production of ketone bodies, the metabolism of bile salts, and possibly the metabolism of cholesterol.

In the present discussion we are not particularly concerned in the first five functions listed above. Interest has been centered on the metabolism and storage of food in the liver. In an interesting review article, Mann (1937) discusses the "role of the liver as the commissariat of the body", and summarizes the most significant facts concerning hepatic activity in maintaining the food supply to the body tissues. It is the duty of the liver to provide a constant stream of food material to the other tissues. Mann discusses this function in relation to the storage of food materials, manufacture of food, and mechanisms of regulation. These functions will be described very briefly in reference to the three main classes of foodstuffs.

Proteins leave the digestive tract in the form of amino acids and are carried to the liver by way of the portal vein. There they are stored temporarily, then a

certain proportion of these are built into body tissue; the excess deaminized by the liver, and the remaining parts burned or converted into carbohydrates. Bollman, Mann, and Magath (1926) demonstrated that deaminization is dependent on the liver since it did not occur after the liver was removed. The ammonia split off during deaminization is converted into urea. That urea formation is the exclusive function of the liver, was proved by Bollman, Mann, and Magath (1924) in a very clever series of experiments on dogs, involving the removal of kidneys and liver. Schmidt and Allen (1938) indicate that the Krebs and Henseleit theory of the mechanism of urea formation is quite generally accepted. Chemical reactions between ornithine, carbon dioxide, ammonia, and water, brought about by actions of enzymes, including argenase, form the basis of the theory. These workers built their conclusions upon observations of reactions occurring in tissue slices placed in appropriate physiological solutions. Of the tissues studied, the liver only was capable of forming urea.

It was believed by Pflüger that there was a special form of storage protein which corresponded to glycogen as the storage form of carbohydrate. But Luck (1936), in a study of rats reared on high and low protein diets, concluded that none of the liver proteins can be singled

out as a reserve of labile protein, and that all participate equally in the storage of proteins.

The liver is known to play an important part in fat metabolism and to be important as a fat depot. The deposition of fat in the liver may assume pathological importance. Campbell (1940) has outlined the literature in respect to the production, the cure, and the prevention of fatty livers.

Fatty acids, coming from the fat stores of the body or from the food fed, are transported to the liver by the blood. One of the first steps in their utilization is believed to be a desaturation, since the iodine numbers of the liver fat are higher than those of the source fatty acids (Leathes and Raper, 1925). They may then be converted into phospholipids, and after desaturation and phosphorylation are oxidized. The beta oxidation theory of Knoop probably explains the principle mode of oxidation (Sherman, 1938). Mann says in his discussion of fat metabolism:

> "1. The exchange of fat in the liver is an extremely important process;
> 2. It is difficult of investigation; and
> 3. Some progress is being made but not commensurate to the importance of the subject."

The relation of the liver to the metabolism and storage of carbohydrates is of special interest in the present study. Claude Bernard, the great French physiologist, first traced carbohydrates through the labyrinths of metabolism (Olmsted, 1938). His basic demonstration proved that glucose is present in the blood. Then in a classic experiment, Bernard, after feeding a dog meat, found that blood from the portal vein contained no sugar but that blood from the hepatic vein did. He concluded that the glucose must have come from the liver and in 1850, published a paper in the Proceedings of the Academy of Sciences (of France) in which he announced a new function of the liver to which, later, he gave the name glycogenesis. He termed the hypothetical storage substance, glycogen. By March, 1857, Bernard gave directions for isolating glycogen, which are practically the same as those used today. Although all the theories advanced by Bernard are not accepted today, certain clean-cut facts are the foundations of our present knowledge of carbohydrate metabolism. These principal facts are: sugars are carried to the liver in the portal blood, excess carbohydrate is stored in the liver as glycogen, is released again into the blood as glucose and finally oxidized to carbon dioxide and water.

Sugar is also stored in the muscles as glycogen but

the percentage composition of the liver is higher and only the glycogen of the liver is capable of being transformed into glucose and mobilized for the use of the tissues. It is interesting that even in the absence of a dietary source of carbohydrate, glycogen is present in the liver. The liver has the capacity to use body protein and fat as a source of glycogen.

The amount of glycogen present in the liver is extremely variable and changes occur rapidly. Little is known about the actual chemical processes taking place in the liver cells which change glucose to glycogen (glycogenesis) and which transform glycogen back to glucose (glycogenolysis). Enzymes are no doubt involved in these reactions. It is evident that there are extremely delicate mechanisms which regulate these transformations and supply a constant stream of glucose to the tissues of the body. When the liver is removed, hypoglycemia results and the animal dies if glucose is not administered.

It is known that hormones secreted by the endocrine glands have to do with the metabolic processes taking place in the liver. Much research is being conducted in an effort to make clear the complexities of the relationships involved. Mering and Minkowski (1890) in a classic piece of work on depancreatized dogs, discovered that removal of the pancreas results in hyperglycemia and

glycosuria. Later, Banting and Best (1922) found that a hormone which they called insulin is secreted by the pancreas and that this hormone has to do with the regulation of carbohydrate metabolism. The injection of insulin restores to the depancreatized animal its ability to utilize sugars and glycogen is deposited in the liver. If insulin is administered to the normal animal there is a fall in blood sugar.

Injection of epinephrine, on the other hand, causes increased glycogenolysis and the effect is hyperglycemia. Britton and Silvett (1932) believe that removal of the adrenals is followed by hypoglycemia and a reduction of glycogen in muscle and liver, but Mann says such findings have only occasionally been noted in his adrenalectomized dogs. There is apparently an antagonism in the effects of insulin and epinephrine. In an experiment with human subjects, Asmussen, Wilson, and Dill (1940) found that during muscular exercise the administration of epinephrine increased the blood sugar level, insulin decreased it, and injections of both caused small fluctuations. Cori and Cori (1928a, 1928b, 1928c, 1930) have reported the results of an intensive study of the mechanisms of epinephrine action in which they concluded that decreased utilization of blood sugar plays an important part in epinephrine hyperglycemia and that insulin is an

inhibitor of the hepatic glycogencolysis accelerated by epinephrine. Since it is believed that there is an increased output of epinephrine during emotional states, fear and excitement should result in increased glycogenolysis and thereby cause a variation in the glycogen content of the liver.

More recently there has been mentioned a relation between the hormones of the anterior pituitary and carbohydrate metabolism. It has been shown that when the pancreas is removed in a dog, the resulting diabetes will disappear if the anterior pituitary is also removed. Marks and Young (1938) demonstrated that rabbits receiving injections of anterior pituitary extract during a 24-hour fast showed higher concentrations of glycogen in the liver and muscles, than did the controls. Russell (1936) stated that hypophysectomized rats lose body carbohydrate at a greater rate than do normal animals. The hormone of the pituitary gland having the effect of raising the blood sugar level is sometimes called the diabetogenic hormone (Wolf, 1940) and there has even been postulated a pancreatropic hormone whose action is antagonistic to the diabetogenic one.

Mann says that hepatic activity can be changed by hormones by effecting: (1) the rate of body metabolism, (2) the intrinsic mechanism of the liver, either for

storage or manufacture of food, and (3) the amount of available food materials reaching the liver.

Various factors besides the action of hormones affect the amount of glycogen found in the liver at a given time. Some of these factors are: the amount and character of food ingested, extent of bodily exertion, sex, species of animal, and the diurnal cycle.

The amount of glycogen stored in the liver can be varied greatly depending on the quantity and quality of the food ingested. It is possible, by continuous intravenous injections, to increase the stores of glycogen in the liver, far above the normal limit. Dukes (1937) says that in dogs, heavy carbohydrate feeding may increase the percentage composition of glycogen in the liver from 3 or 4 per cent to 12 per cent. Butsch (1932) found that the liver of dogs could store glycogen up to 20 or 25 per cent.

MacKay and Bergman (1933) measured the rate of glucose absorption and glycogen deposition in the livers of rats fed glucose after having been reared on diets high either in carbohydrates, fat, or protein. They found that hepatic glycogen was synthesized as rapidly on all three diets. They concluded that the pre-experimental diet had no effect after 24 and 48 hours of starvation upon the rate of glycogen deposit in the liver after

feeding glucose. Greisheimer and Johnson (1930) fed test diets containing 87 per cent of the calories in the form of sucrose, lard, or casein to rats. The animals were killed without starvation and the concentration of glycogen in the liver determined. While the quantity of the carbohydrate was significantly higher in rats on the high sucrose diet, it was lower on lard and casein than on the control diet. A close relationship was found between the quantity of food making up the last 12 hours of food intake and the liver glycogen content. Glycogen is probably not readily formed on a high lard diet.

It is well known that increased energy requirements due to bodily exertion result in depletion of the glycogen stores of the body. As Howell (1927) says, workers agree that glycogen disappears from a muscle in proportion to its functional activity. Since muscle glucose is replaced by glucose from the blood, which results in large part from the breakdown of glycogen in the liver, liver stores of glycogen are also depleted by muscular activity. Various procedures have been adopted experimentally to deplete the muscles or liver of their glycogen. Nutter (1941) worked out a system to fatigue rats, thereby lowering the stores of glycogen in the liver, to secure a base-line value for the recovery of glycogen in further experiments. The rats were forced to

swim until exhausted (a period of about 10 hours). It was found that while fatiguing exercise during a period of fasting decreased the muscle glycogen to much lower levels than fasting alone, it reduced the liver glycogen only slightly more than fasting alone. Other forms of exercise, such as running in revolving cages, running on a treadmill, or muscular contractions caused by electrical stimulation, are sometimes used as experimental procedures.

Greisheimer (1931) pointed out that male rats fed on various diets had higher liver glycogen and lower liver fat than did females on the same diets. Deuel et al have investigated this sexual variation in a series of experiments (1934, 1937). They found that the concentration of glycogen in the liver of male rats was higher than in females for all periods of fasting up to 96 hours after administration of glucose. In the earlier work (1934), no differences were found in unfasted male and female rats. In another study (1937) with a large group of rats on high fat diets, the relative concentration of liver glycogen in the female averaged about 60 per cent of that in the males. In normal and adrenalectomized rats, the same laboratory (1937) reported a sex difference in the formation of glycogen following administration of glucose, and that the rate of glucose absorption is higher in the

female than the male. They investigated the effect of age upon the sexual variation in the content of glycogen in the liver and found no sexual difference in rats 28 to 30 days of age nor in old rats (17 to 24 months). With the exception of these groups, the values were consistently lower in the female than in the males. Since the effect is not observed in the old or young animal, Deuel and coworkers believe that the alteration is referable to the sex glands. They believe that there may be an inhibitory glycogenic effect caused by the ovary rather than a stimulatory effect on glycogenesis traceable to a testicular secretion in the male. The higher food consumption by the males was not responsible for the difference since it occurred when no variation in food intake on the weight basis could be shown.

There is also a difference in the content of glycogen in the liver in normal animals of various species. Dukes (1937) gives the following values: rabbit, 5 to 7 per cent; dog, 3 to 4 per cent; ewe (pregnant), 3.8 per cent. Marble, Grafflin, and Smith (1940) in an investigation presenting data on normal values of glycogen in the liver of guinea pigs, found averages of 6.16 and 6.98 per cent in two series of male animals. Apparently the relative amount of glycogen in the liver of normal guinea pigs is

higher than in rats. (See Table 6). A cyclic variation in hepatic activity has sometimes been described. Åsgren, Wilander, and Jorpes (1931) reported changes in the glycogen content of the livers of well-fed mice, which they believed to be independent of the intake of food, but to be dependent upon "astronomic factors". The livers of these mice contained more glycogen during the night, than during the day. Higgins, Berkson, and Block (1933) further investigated this so-called diurnal effect, by training rats to eat at various two-hour periods during the twenty-four hours, then killing the animals six hours after eating 10 gm. of food and determining the concentration of glycogen in the liver. Under these circumstances, the diurnal variations were not observable and these workers believe that cyclic changes in the livers of rats whose consumption of food is rigidly controlled are dependent on the physiological factors associated with feeding.

On the other hand, Deuel and coworkers (1938) observed a diurnal variation in the level of glycogen in the livers of unfasted rats having access to a stock diet. The liver glycogen in males varied from 4.74 per cent at 4 a.m. to 1.68 per cent at 4 p.m. A similar cycle was noted with females, although the peaks were delayed. These variations, however, may have been due to the

periodicity of food consumption since the rat is a nocturnal feeder. When a glucose test meal was given after a fasting period and the determinations of glycogen made after 12 hours, a very constant level was noted for each sex at the 4-hour intervals studied throughout the day. These workers also conclude that the diurnal variations are of dietary origin.

The relation between the fat and glycogen content of livers is of special interest in the present study. Rosenfeld's comments (1902) on the antagonism between fat and glycogen have already been discussed. Mann (1937), says that since changes in fat content of the liver occur more slowly than those in glycogen content, it is possible for the liver to have both a high glycogen and fat content. In general, when the content of one is increasing, the other is decreasing, so they may be said to have a reciprocal relationship to each other. Goodale (1937) described a patient who died of hypoglycemia following the destruction of glycogen stores of the liver by fat infiltration due to excessive alcoholism. He says that when the glycogen storage function of the liver is impaired, fat infiltration is increased. This is in agreement with Rosenfeld who suggests that livers with a high glycogen content refuse to take on fat and that

the antagonism between fat and glycogen protects man from the systematic appearance of fatty livers.

Bodansky and Bodansky (1940) mention several cases of fatty metamorphosis of the liver and its relation to hypoglycemia. One patient showed severe hypoglycemia which could not be relieved by administration of glucose. A study of a small piece of liver removed by biopsy, showed fatty metamorphosis. An infant exhibiting hypoglycemia before death, showed fat replacement in the liver cells and no glycogen. The authors in summarizing, say that hypoglycemia is the outstanding finding in cases of fatty infiltration of the liver.

There is some evidence that there is a disturbance of carbohydrate metabolism in the toxemias of pregnancy. Titus and Dodds (1928) investigated the theory that carbohydrate deficiency may be the chief factor in the causation of pregnancy toxemias. They found fluctuations in the blood sugar level during eclampsia and believe that eclamptic convulsions are hypoglycemic reactions. The authors also state that the tendency to low blood sugar values in patients with <u>hyperemesis gravidarum</u> indicate glycogen depletion and that the lowest values indicative of the most profound glycogen depletion were seen in the sickest patients.

Siegel and Wylie (1933) investigated the claims of

Titus and Dodds and found that while fluctuations in blood sugar in eclampsia with a tendency to lower blood sugar levels occurred after each convulsion, the majority of patients were hyperglycemic. However, in preeclampsia low or subnormal blood sugar was characteristic. Siegel and Wylie also believe that eclampsia is accompanied by unstable carbohydrate metabolism. In disagreement with the preceding reports, Mays and McCord (1935) state that neither hypo- nor hyperglycemia is characteristic of eclampsia and that the absolute blood sugar concentration has no effect whatever on the incidence of convulsions. Rowe et al (1936) present the results of a study of carbohydrate metabolism in 50 pregnant women, exhibiting some form of toxemia. In toxic pregnancy, there was no greater incidence of glycosuria than in normal pregnancy. Toxemia produced no change in the blood sugar levels, but a study of sugar tolerance indicated that toxic pregnancy produced marked change in galactose and levulose metabolism.

It will be noted that there is lack of agreement concerning the state of carbohydrate metabolism in the toxemias of pregnancy, but the possibility certainly exists that derangement may occur.

From the experimental standpoint, there have been

very few studies dealing with carbohydrate metabolism in abnormal pregnancy. However, the following studies are of importance in this connection.

In an investigation of the pregnancy disease of ewes, Roderick, Harshfield, and Merchant (1933) compared the concentration of glycogen in livers of diseased ewes to that in normal control ewes. The glycogen in the liver was reduced from an average of 3.76 per cent in normal ewes to 0.31 per cent in ewes with the pregnancy disease. The lowering of the liver glycogen resulted in a frequent lowering of the blood sugar level. Roderick and coworkers feel that injury to the liver, with its deposition of fat, results in an inability to store glycogen and exhaustion of the endogenous supply of carbohydrate necessary to protect the animal from acidosis. Hypoglycemia is correlated with a loss of liver function.

In a further study of the pregnancy disease of sheep, Roderick and coworkers (1937) produced acetonuria and acidosis and other symptoms of the pregnancy disease by withholding food from pregnant ewes. Fatty livers were found in starved ewes and lambs. The authors conclude that pregnancy disease is definitely related to carbohydrate metabolism, and that with an inadequate

carbohydrate intake, glycogen is withdrawn from the liver, and fat takes its place. They mention unpublished data to the effect that fetal lambs store glycogen prior to parturition, and that as high as 9 per cent of glycogen has been found in the fetal livers--a value two to three times higher than that present in the maternal liver. Fatty livers, induced by starvation, were cured by feeding the animals until ketosis disappeared. As Roderick says, this demonstrates the reversibility of the reaction between the presence of glycogen and fat in the livers of sheep.

RESULTS AND DISCUSSION

RELATIVE CONCENTRATION OF GLYCOGEN IN MATERNAL LIVERS

SERIES I

Steenbock V Virgins vs. Pork I Virgins

The quantity of glycogen present in the livers of virgin rats fed the two test diets was first studied. The concentration of liver glycogen was determined in a group of virgin rats reared on the stock diet, designated as Steenbock V, and in a group of virgin rats reared on the experimental diet, Pork I. These animals, after a 15-hour fast were offered 4 gm. of the diet on which they had been maintained, and the tissues removed for analysis after a seven-hour interval. Analytical results, weights and ages of the animals, and liver weights are presented in Table XI and Table XII (Appendix) and are summarized in Table 6. The two groups were very similar in regard to age and body weight at the time the determinations were made. The liver weights, total quantities of glycogen in the liver, and percentages of glycogen were compared by means of a statistical analysis (Table 7).

TABLE 6. AVERAGE CONCENTRATION OF GLYCOGEN IN LIVER - SERIES I AND II

Diet	Reproductive status	Number of rats	Wt. of rats	Age of rats	Wt. of liver	Wt. of liver glycogen	Per Cent of glycogen
		gm.	gm.	days	gm.	mg.	
Pork I	Virgin	8	173.7	152.1	5.6998	231.69	4.08
	Pregnant	26	236.0	142.6	7.5217	193.24	2.57
Steenbock V	Virgin	8	178.8	149.5	5.9856	213.11	3.58
	Pregnant	23	262.9	146.9	7.7434	246.20	3.18
Pork I (Series II)	Pregnant	10	251.4	117.4	8.6367	201.60	2.34

TABLE 7. ANALYSIS OF VARIANCE OF WEIGHTS OF LIVER AND GLYCOGEN IN LIVERS OF VIRGIN RATS FED DIFFERENT DIETS - SERIES I

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Weights of livers	Items within the groups	14	8.6088	0.6149	
	Between group means	1	0.3268	0.3268	
	Total	15	8.9356		
Absolute quantity of glycogen (mg.)	Items within the groups	14	14121.25	1008.66	
	Between group means	1	1381.60	1381.60	
	Total	15	15502.85		1.37
Relative quantity of glycogen (per cent)	Items within the groups	14	2.0826	0.1488	
	Between group means	1	1.0100	1.0100	
	Total	15	3.0926		6.79*

TABLE 8. CONCENTRATION OF GLYCOGEN IN LIVERS OF NORMALLY-FED RATS AS REPORTED BY VARIOUS INVESTIGATORS

Investigators	Date	Number of animals	Concentration of liver glycogen	Remarks
			per cent	
Greisheimer	1930	6F and 4M	4.49	Stock diet, no starvation
Greisheimer	1931	3F	5.20	Starved 48 hrs., fed stock diet for 24 hrs.
Cori, Cori, Buchwald	1930	8	2.44	Had been given glucose but were called "well fed"
Deuel, Gulick, Grunewald, Cutler	1934	20F	3.53	Stock diet, no starvation
Hrubetz, Dotti	1934	112	1.78	Fed normally
Deuel, Butts, Hallman, Murray, Blunden	1937	10F 6F 20F	2.39 1.79 3.80	13-76 days old 88-90 days old 17-18 mo. old Unfasted, stock diet
Mutter	1941	4F	2.78	Animals taken directly from the stock colony

The F test of significance was used.[1] (One star indicates that F is significant, two stars highly significant.) More variation in liver weights occurred within the groups than between the groups, so there was no real difference in the weights of this organ in the two groups. However, the livers of the pork-fed rats contained a significantly higher per cent of liver glycogen than did the livers of the virgin control rats. From previous studies it is known that the livers of the pork-fed virgins are fatty. Could the presence of this fat influence and retard in some way the progress of glycogenolysis, resulting in higher liver stores even in the pork-fed virgins?

The virgin rats used in the present study and treated in the manner described are probably comparable with normally-fed female rats mentioned in the literature. Some values for the concentration of liver glycogen in such rats reported by other investigators are compiled in Table 8. It must be borne in mind that age, sex, quality of diet, and environmental conditions may have been variable in the studies reported. However, the values for the per cent of liver glycogen, as determined in the study herein reported, fall within the range of

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1. Snedecor, G. W.
1938. Statistical Methods, revised ed., Collegiate Press Inc., Ames, Iowa, pp. 184-187.

those presented by other workers. They compare very closely with the figures given by Deuel et al (1934, 1937).

Steenbock V Pregnant Rats vs. Pork I Pregnant Rats

Liver glycogen was also determined in pregnant rats reared on the stock diet and in pregnant pork-fed rats. These groups were fasted, then fed seven hours before killing, in the manner previously described. Analyses were performed on the twenty-first day of pregnancy. The experimental data are presented in Tables X and XII (Appendix) and the mean values are summarized in Table 6. Upon autopsy, 11 of the animals fed the pork diet exhibited total resorptions. For this reason weights of uteri and feti are not complete in Table XII. This may also account for the fact that on the whole the control animals were somewhat heavier than the experimental rats, although they were of approximately the same age. The absolute quantities of glycogen produced by the control group and the rats fed the pork diet were 246.2 gm. and 193.2 gm. respectively; the relative amounts, 3.18 per cent and 2.57 per cent. The weights of glycogen in the liver, the percentages of liver glycogen, and the liver weights in each group were compared statistically using the F test of significance (Table 9). As in the

TABLE 9. ANALYSIS OF VARIANCE OF WEIGHTS OF LIVER AND GLYCOGEN IN LIVERS OF PREGNANT RATS FED DIFFERENT DIETS - SERIES I

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Liver weights	Items within the groups	47	29.0125	0.6172	
	Between group means	1	0.5985	0.5985	
	Total	48	29.6110		
Absolute quantity of glycogen (mg.)	Items within the groups	47	99,704.0878	2,121.3635	
	Between group means	1	32,964.6973	32,944.6973	** 15.53
	Total	48	132,648.7851		
Relative quantity of glycogen (per cent)	Items within the groups	47	15.6298	0.3325	
	Between group means	8	4.4312	4.4312	** 13.33
	Total	48	20.0610		

preceding analysis, the variation in liver weights within the groups was greater than that between the groups. Liver glycogen both from the relative and absolute standpoints in the Steenbock-fed group was higher than that in the pork-fed group and the differences were highly significant. On the basis, then, of the quantity of glycogen stored, the experimental animals showed either a less efficient storage of carbohydrate or a higher rate of glycogenolysis. Possible explanations for the difference in liver composition will be pointed out later.

No investigations reporting the quantity of glycogen present in the liver of pregnant rats were found in the literature.

Virgin Rats vs. Pregnant Rats

A comparison of the store of glycogen in the liver of virgin rats with that of pregnant rats is interesting (Table 10 and Table 11). In both the control and experimental groups, the weights of the livers of pregnant rats were significantly higher than those of virgins. The absolute weight of liver glycogen in the virgin animals fed the pork diet was greater than that in the pregnant rats but the variance was not significant. However, the

TABLE 10. ANALYSIS OF VARIANCE OF WEIGHTS OF LIVER AND GLYCOGEN IN LIVERS OF PREGNANT AND VIRGIN PORK-FED RATS - SERIES I

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Liver weights	Items within the groups	32	22.6390	0.7081	
	Between group means	1	20.3044	20.3044	
	Total	33	42.9634		** 28.67
Absolute quantity of glycogen (mg.)	Items within the groups	32	75,863.67	2,370.74	
	Between group means	1	9,045.65	9,045.65	
	Total	33	84,909.32		3.82
Relative quantity of glycogen (per cent)	Items within the groups	32	11.7129	0.3660	
	Between group means	1	13.9151	13.9151	
	Total	33	25.6280		** 38.02

TABLE 11. ANALYSIS OF VARIANCE OF WEIGHTS OF LIVER AND GLYCOGEN IN LIVERS OF PREGNANT AND VIRGIN CONTROL RATS - SERIES I

Analyses made	Source of variation	Degrees of freedom	Sum of Squares	Mean square	F
Liver weights	Items within the groups	29	14.9606	0.5159	
	Between group means	1	18.3385	18.3385	**
	Total	30	33.2991		35.55
Absolute quantity of glycogen (mg.)	Items within the groups	29	37,961.66	1,309.02	
	Between group means	1	6,111.70	6,111.70	**
	Total	30	44,073.36		4.67
Relative quantity of glycogen (per cent)	Per cent of liver glycogen within the groups	29	5.9995	0.2069	
	Group means	1	0.9644	0.9644	**
	Total	30	6.9639		4.66

weight of glycogen was significantly higher in the pregnant control rats than in the virgin animals fed the same diet. In the case of rats reared on both diets the percentage of glycogen was greater in the livers of the virgins then in the pregnant animals and the differences were highly significant.

The difference between the percentage concentration of glycogen in the livers of the pork-fed virgins and the pork-fed pregnant rats was greater than that between control virgins and control pregnant animals. These data studied in connection with the absolute quantity of glycogen present in the livers of each group indicate that pregnancy in the rats given the Pork I diet definitely inhibits the deposition of glycogen to the extent that occurs normally.

Pork I Rats with Total Resorptions vs. Pork I Rats with Living Feti

The frequent occurrence of complete resorptions in this particular group of pork-fed pregnant rats gives rise to interesting speculations. Does the occurrence of resorption result in metabolic disturbances which cause depletion in liver stores? Or may toxins be released which have an effect on carbohydrate metabolism?

Conversely, did disturbances of carbohydrate metabolism in the pork-fed groups result in the poor nutrition and consequent death of the feti? In an attempt to answer some of these questions, the pork-fed females were sorted into two groups, those showing total resorptions and those with living feti. The liver weights, weights of glycogen in the liver, and percentages of glycogen in the liver are shown in Table XVI (Appendix) and the means shown in Table 12.

TABLE 12. AVERAGE CONCENTRATION OF LIVER GLYCOGEN IN RESORBING AND NON-RESORBING PORK-FED FEMALES

Condition of female	Number of rats	Wt. of the liver	Wt. of glycogen in the liver	Per cent of glycogen in the liver
		gm.	mg.	
Total resorptions	11	7.1158	164.16	2.31
Living feti	14	7.7568	213.31	2.78

The average liver weight in the animals with living feti was significantly higher than in those with resorptions (Table 13). The weight of glycogen in the liver was also significantly higher but the difference in percentage of liver glycogen was not significant. In this case it appears that the increased liver stores were at least partly due to the increased size of the liver. The average percentage of liver glycogen in the females with

TABLE 13. ANALYSIS OF VARIANCE OF LIVER WEIGHTS AND GLYCOGEN IN LIVERS OF PORK-FED RATS WITH RESORPTIONS AND THOSE WITH LIVING FETI

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Liver weights	Items within the groups	23	12.1617	0.5288	
	Between group means	1	2.5318	2.5318	
	Total	24	14.6935	14.6935	* 4.79
Absolute quantity of glycogen (mg.)	Items within the groups	23	47,855.1207	2,080.6574	
	Between group means	1	14,878.9633	14,878.9633	
	Total	24	62,734.0840		* 7.15
Relative quantity of glycogen (per cent)	Items within the groups	23	9.3379	0.4060	
	Between group means	1	1.3477	1.3477	
	Total	24	10.6856		3.32

living feti, i.e., 2.78, was also significantly lower than that for the pregnant control rats, 3.18 per cent, but some of the difference observed in the original analysis has been removed by the elimination of the females with resorptions from the Pork I group (df, 35 and 1; F, 4.57).

Whether or not partial resorptions were responsible for the lower amount of glycogen present in the livers of the pork-fed rats was next investigated. The average number of partial resorptions per experimental rat was 1.6 in contrast to 0.8 in the control group. When resorptions in individual rats in each group were studied in relation to liver glycogen it was found that there was no consistent lowering of glycogen in maternal livers due to the number of individual resorptions. In fact, the average amount of glycogen (both absolute and relative) in the liver of pork-fed rats with only living young in the uteri was lower than the average amount present in a similar group of Steenbock-fed rats.

Liver Glycogen in Terms of 100 gm. of Female

As another check on the observation that a sub-normal amount of glycogen is stored in livers of rats fed the pork diet, variation due to the differences in body

weight was next considered. The quantities of liver glycogen in pregnant control and pregnant experimental animals of Series I were calculated in terms of mg. of glycogen per 100 gm. of rat (Table XIV, Table XV, Appendix).

The number and weights of feti present were extremely variable and consequently influenced the body weights. For example, 11 of the pork-fed rats that had been pregnant, contained no developed feti and one mother had a single fetus. The weights of the intact uteri varied from 10.7 gm. to 58.5 gm. in the group fed the pork diet and from 41.5 gm. to 74.6 gm. in the control rats. In a further attempt to reduce the effect of this variation, the quantities of glycogen present in the livers of both groups were also calculated in terms of mg. of glycogen per 100 gm. of female minus the weight of uterus (Table XIV, XV, Appendix).

The mean values for each group of rats obtained by the two methods of calculation are given in Table 14. Animals for which data were incomplete due either to resorptions or experimental error, were omitted in the calculation of these means.

TABLE 14. AVERAGE WEIGHT OF GLYCOGEN IN CONTROL AND PORK-FED RATS OF SERIES I CALCULATED IN TERMS OF BODY WEIGHT

Diet	No. of rats	No. of feti	Wt. of liver	Wt. of glycogen per 100 gm. of gravid female	Wt. of glycogen per 100 gm. of female minus uterus
			gm.	mg.	mg.
Pork I	15	7.9	7.7732	86.25	103.52
Steenbock V	22	10.8	7.8080	93.93	120.89

Analysis of variance (Table 15) showed that again, there was more variation in liver weights within the groups than between the groups. The weight of glycogen per 100 gm. of gravid female was higher in the control group than in the pork-fed group but not significantly so. However, when the weight of the uterus was subtracted, the variance between the groups was significant.

RELATIVE CONCENTRATION OF LIVER GLYCOGEN - SERIES II

It was thought that the quality of the 4 gm. test meal given to the rats after the 13-hour fast might be related to the low glycogen stores in the livers of the females fed the pork diet. Consequently, a group of pregnant pork-fed rats was starved for 13 hours, then offered 4 gm. of the Steenbock ration instead of the

TABLE 15. ANALYSIS OF VARIANCE OF WEIGHTS OF LIVER AND GLYCOGEN IN LIVERS OF PREGNANT CONTROL AND PORK-FED RATS IN TERMS OF BODY WEIGHT

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Liver weights	Items within the groups	35	18.7836	0.5367	
	Between group means	1	0.0110	0.0110	
	Total	36	18.7946		
Mg. of glycogen per 100 gm. of gravid female	Items within the groups	35	12,615.9074	345.4960	
	Between group means	1	523.5481	523.5481	
	Total	36	12,615.9074		1.52
Mg. of glycogen per 100 gm. of female minus uterus	Items within the groups	35	18,753.4142	535.8118	
	Between group means	1	2,689.5225	2,689.5225	
	Total	36	21,442.9367		* 5.02

pork mixture. These animals were otherwise treated exactly like the pregnant pork-fed rats of Series I. Data obtained are shown in Table XVII (Appendix) and the means, in Table 6. Results of an analysis of variance are presented in Table 16. The variances between the weights of glycogen and the percentages of glycogen in the two groups were not significant. The quality of the test meal, therefore, did not influence glycogen storage in the pork-fed rats, and in later discussion, data relating to the animals of Series II can be included with those obtained from the pork-fed females of Series I.

The weights of the livers of the rats in Series II were significantly higher than those of the corresponding group in Series I. No explanation can be offered unless the presence of one or two exceptionally heavy livers in the smaller group raised the average. For example, the liver of rat number 25848 weighed 10.2755 gm.

ANALYSIS IN TERMS OF BOTH FETAL AND MATERNAL LIVER GLYCOGEN - SERIES I AND II

Fetal Livers

A study of the data shows that the quantity of

TABLE 16. ANALYSIS OF VARIANCE OF WEIGHTS OF LIVER AND GLYCOGEN IN LIVERS OF RATS OF SERIES II AND PREGNANT PORK-FED RATS OF SERIES I

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Liver weights	Items within the groups	34	22.3457	0.6572	
	Between group means	1	8.9780	8.9780	
	Total	35	31.3237		13.66**
Absolute quantity of glycogen (mg.)	Items within the groups	34	85,529.8666	2515.5840	
	Between group means	1	504.3403	504.3403	
	Total	35	86,034.2069		
Relative quantity of glycogen (per cent)	Items within the groups	34	13.1894	0.3879	
	Between group means	1	0.3990	0.3990	
	Total	35	13.5884		1.03

glycogen deposited in the maternal liver was only part of the picture. In pregnancy, the concentration of glycogen stored in fetal livers as well as in maternal should be considered. Fetal livers were removed from part of the pregnant control rats of Series I, from two of the pork-fed pregnant rats of Series I, and from all of Series II. Since, as has been shown, these two last groups were comparable, the feti from Series II may be considered as typical of pork-fed rats. The livers from each litter were pooled, as has been described, and the glycogen determined. The concentration of the glycogen in fetal livers alone, will be discussed first. The analytical data, weights of fetal livers, weights of feti, and numbers of feti are presented in Tables XVIII and XIX (Appendix). The means are shown below.

TABLE 17. AVERAGE CONCENTRATION OF LIVER GLYCOGEN IN THE FETI OF CONTROL AND PORK-FED RATS

Diet	No. of cases	No. of feti	Wt. of feti + placentae	Wt. of fetal livers	Wt. of glycogen	Per cent of glycogen
			gm.	gm.	mg.	
Pork I	12	10.2	48.5	1.6505	95.54	5.92
Steenbock V	11	11.0	54.2	1.9994	119.18	6.10

The statistical analyses of the differences observed

in liver weights, weights and percentages of glycogen are reported in Table 18. By the F test, the fetal livers from the control animals weighed significantly more than those from the experimental animals. The variance between total weight of fetal glycogen produced by the control rats and that produced by the rats fed the pork diet was highly significant. The mothers raised on the stock diet, therefore, were able to produce more fetal hepatic tissue and thereby store more fetal liver glycogen. Since the percentages of glycogen did not vary significantly it is evident that the greater liver stores in the control animals were due to the greater mass of liver tissue.

Very little information is available on the normal concentration of glycogen in fetal livers. Deuel et al (1937) found about 1 per cent of liver glycogen in the "newest" feti observed and 6 per cent in new born rats. The latter would correspond closely to the 21-day old feti analyzed in the present study, and the percentage composition is very similar.

Fetal and Maternal Livers

Maternal and fetal liver glycogen in the two groups of rats have been considered separately. In this section, the total amount of glycogen deposited has been

TABLE 18. ANALYSIS OF VARIANCE OF WEIGHTS OF LIVER AND GLYCOGEN IN FETAL LIVERS OF CONTROL AND PORK-FED RATS

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Liver weights	Items within the groups	21	2.5257	0.1203	
	Between group means	1	0.6986	0.6986	*
	Total	22			5.81
Absolute quantity of glycogen (mg.)	Items within the groups	21	4058.9733	193.2844	
	Between group means	1	3208.1025	3208.1025	**
	Total	22	7267.0758		16.60
Relative quantity of glycogen (per cent)	Items within the groups	21	21.9184	1.0437	
	Between group means	1	0.1865	0.1865	
	Total	22	22.1049		

determined by calculating the weight of maternal glycogen plus fetal glycogen in terms of 100 gm. of maternal and fetal liver and in terms of 100 gm. of gravid female. Results are shown in Tables XX and XXI (Appendix) and means in Table 19. Results of the statistical analysis are shown in Table 20.

TABLE 19. AVERAGE CONCENTRATION OF MATERNAL AND FETAL GLYCOGEN IN CONTROL AND PORK-FED RATS

Diet	No. of rats	Wt. of fetal + maternal liver	Wt. of fetal + maternal glycogen	Maternal + fetal glycogen in terms of 100 gm. of fetal + maternal liver	Maternal + fetal glycogen in terms of 100 gm. of gravid rat
		gm.	mg.	mg.	mg.
Pork I	12	10.1018	306.60	3060.97	122.68
Steenbock V	11	10.0553	380.07	3801.33	138.03

The variance between the total weights of liver tissue produced by the two groups was highly significant as was the variance when the weight of glycogen was expressed in terms of weight of liver tissue. In terms of 100 gm. of rat, the variance was not so great but was significant.

TABLE 20. ANALYSIS OF VARIANCE OF MATERNAL AND FETAL GLYCOGEN OF CONTROL AND PORK-FED RATS

Analyses made	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Weight of maternal and fetal glycogen (mg.)	Items within the groups	21	30,667.90	1,460.38	
	Between group means	1	30,977.61	30,977.61	**
	Total	22	61,645.51		21.21
Total glycogen in terms of total liver tissue (mg. per 100 gm.)	Items within the groups	21	3,926,833.38	186,992.06	
	Between group means	1	3,145,795.74	3,145,795.74	**
	Total	22	7,072,629.12		16.82
Total glycogen in terms of 100 gm. of rat (mg. per 100 gm.)	Items within the groups	21	4,337.2134	206,5340	
	Between group means	1	1,353.6568	1,353.6568	*
	Total	22	5,690.8702		6.55

Pork I and Steenbock V Rats Matched as to Number of Feti

The number of feti varied so much from one female to another that it seemed possible that variations in maternal glycogen might be a reflection of different fetal demands. Whether the difference in the quantity of liver glycogen laid down by the experimental and control rats already demonstrated, holds when the two groups were matched according to the number of feti present, was next determined. Results are shown in Table XXII (Appendix). It will be observed that in every case except one, maternal livers of Steenbock-fed rats contained a higher percentage of glycogen than did those of the pork-fed animals with the same number of feti. It is interesting that the weights of the feti were not consistently higher in the control group. In this group, it may be noted also that the percentage of fetal glycogen tended to increase as the number of feti decreased. But at the same time the concentration of maternal glycogen was maintained at a more or less constant level. In the experimental group neither of these relations was as consistent. The lowest percentage of glycogen was found in the animal with the greatest number of feti, but otherwise a reciprocal relationship between percentage of

liver glycogen and number of feti was not evident.

From all the analyses made in the preceding sections the data indicate that the pregnant control rats produced significantly larger quantities of glycogen under the experimental conditions imposed then did the pregnant rats fed the pork diet. This was true when the data were considered in terms of maternal livers alone, fetal livers alone, or a combination of fetal and maternal livers. While total resorptions seemed to reduce the average quantity of glycogen stored in the maternal liver, the difference demonstrated between control and experimental rats holds either when the two groups are matched as to number of living feti or when they were compared as to the number of individual resorptions.

RELATIVE ABSORPTION OF GLUCOSE - SERIES III AND IV

The relative absorption of glucose was studied in all groups of rats in Series III and IV by analyses of the reducing substances present in the gastrointestinal tracts at the time of removal of the liver. The quantity of reducing substances normally present in the gastrointestinal tract of starved rats was first determined by analyzing the tracts of virgin and pregnant animals from the control and pork-fed groups after a 13-hour fast.

Results are presented in Tables XXIII and XXIV (Appendix). Next, the quantity of reducing substances present in the tracts of the four groups of rats four hours after the feeding of glucose, was determined (Tables XXV, XXVI, XXVII, Appendix). Data from the analyses of the intestinal contents of all rats in Series III and IV are summarized in Table 21.

In the starved rats, there was no consistent variation in the reducing substances between the virgin and the pregnant animals nor between the control and the pork-fed groups, virgin or gravid. The values obtained in the present study probably represent the average amount of reducing substances normally present in the tracts of starved rats, as may be observed by comparing the figures with those reported by other investigators (Table 22).

By comparing the figures obtained from the starved rats and those fed glucose, it may safely be assumed that absorption of glucose was complete after the four-hour interval. Also, the pork-fed rats exhibited no abnormality in intestinal absorption. The animals killed on the twenty-second day of gestation did not differ in this respect from those killed on the twenty-first day (Table 21).

TABLE 21. AVERAGE WEIGHT OF REDUCING SUBSTANCES PRESENT IN THE GASTROINTESTINAL TRACTS OF RATS IN SERIES III AND IV

Diet	Reproductive status	Post-starvation treatment	Number of rats	Wt. of reducing substances in gastrointestinal tract
				mg.
Pork I	Virgin	No food	5	11.46
	Pregnant	No food	5	13.53
Steenbock V	Virgin	No food	5	12.65
	Pregnant	No food	5	11.51
Pork I	Virgin	Fed glucose	8	12.50
	Pregnant	Fed glucose	8	12.48
Steenbock V	Virgin	Fed glucose	8	12.71
	Pregnant	Fed glucose	8	10.25
Pork I Series IV	Pregnant	Fed glucose	5	12.29

TABLE 22. AVERAGE CONCENTRATION OF REDUCING SUBSTANCES IN THE GASTROINTESTINAL TRACT OF STARVED RATS AS REPORTED BY CERTAIN INVESTIGATORS

Investigators	Date	Number of rats	Quantity of reducing substances in the gastrointestinal tract	Remarks
			mg.	
Cori	1925	3M	15.9 9.0 12.7	Fasted 48 hrs.
Miller, Lewis	1932	17	8.3 - 16.3 Av. 11.2	Fasted 48 hrs.
Farrankop	1941	20F	9.85 - 13.72 Av. 12.13	Fasted 13 hrs.

RELATIVE CONCENTRATION OF LIVER GLYCOGEN - SERIES III AND IV

Starved Rats

To determine the effectiveness of the starvation period in depleting the stores of glycogen in the liver, determinations were made on starved pregnant and virgin animals from both the control and experimental groups. Cori (1926) calls the glycogen present in the livers of such animals "preformed glycogen." Weights of rats, ages, liver weights, and analytical results are shown in Tables XXVIII, XXIX, XXX, and XXXI (Appendix), and the means are summarized in Table 23.

In rats fed both diets, the absolute and relative amounts of glycogen were higher in the virgins than in the pregnant animals. The livers of the Steenbock-fed virgins contained more glycogen than the livers of the virgins fed the pork diet in spite of the fact that the livers of the first group were the lighter. The similarity between the pregnant rats reared on the two test diets is remarkably close. However, all the values observed were very low and it may be assumed that the glycogen in the liver was largely depleted by the 13-hour fast.

TABLE 23. AVERAGE CONCENTRATION OF GLYCOGEN IN LIVERS OF STARVED RATS

Diet	Reproductive status	Number of rats	Age	Body wt.	Wt. of liver	Wt. of glycogen in liver	Per cent of glycogen in liver
			days	gm.	gm.	mg.	
Pork I	Virgin	5	129.6	177.0	5.2190	7.54	0.14
	Pregnant	5	128.8	251.6	7.6414	2.79	0.04
Steenbock V	Virgin	5	150.0	167.6	4.6904	8.97	0.19
	Pregnant	5	136.2	236.0	6.5132	2.87	0.04

A number of reports by other investigators on the concentration of glycogen in the livers of starved rats are compiled in Table 24. The values observed for the virgins in the present study correspond with those reported by several of the investigations, although the starvation period in the present study was only 13 hours long. With the exception of the figure given by Nutter based on a study of three females, none of the percentages are as low as those noted in the pregnant females in this study. Perhaps fetal demands are responsible for the thorough depletion of glycogen stores in the livers of pregnant animals.

Rats Fed Glucose

Since both the quantity and quality of the food given after the starvation period to rats in Series I and II were somewhat variable, a more accurate method of feeding was adopted for Series III and IV. Virgin and pregnant rats reared on both the control and experimental diets were fasted as before, then fed a known quantity of glucose by means of a stomach tube. The weights, ages, liver weights, and results of the glycogen analyses are reported in Tables XXXII, XXXIII, XXXIV, XXXV, and XXXVI (Appendix). Mean values are presented in Table 25.

No difference can be observed in the values for

TABLE 24. CONCENTRATION OF GLYCOGEN IN LIVERS OF STARVED RATS AS REPORTED BY VARIOUS INVESTIGATORS

Investigators	Date	Number of rats	Length of fasting period	Av. quantity of liver glycogen	Remarks
			hr.		
Cori	1926b	7M	48	0.397 per cent	
Barbour, Chaikoff, Macleod, Orr	1927	24 11	24 48	0.16 per cent 0.52 per cent	
Cori, Cori	1928	4	24	6 mg. per 100 gm. of rat	
Cori, Cori	1929	8M	24	0.10 per cent	
Wilson, Lewis	1930		24	0.21 per cent	Values from 0.12 to 0.53 per cent
Gregg	1931	5	48	12.7 mg. per 100 gm. of rat	
Greisheimer	1931	3M 9F	48 48	0.513 per cent 0.137 per cent	
Miller, Lewis	1932	11	24	0.11 per cent	

TABLE 24 (cont.). CONCENTRATION OF GLYCOGEN IN LIVERS OF STARVED RATS AS REPORTED BY VARIOUS INVESTIGATORS

Investigators	Date	Number of rats	Length of fasting period	Av. quantity of liver glycogen	Remarks
			hr.		
MacKay, Bergman	1933	3M	24	0.88 per cent	(Previously on
		3M	48	0.29 per cent	(high fat diet
		3M	24	0.90 per cent	(Previously on
		3M	48	0.45 per cent	(high protein diet
		3M	24	1.05 per cent	(Previously on
		3M	48	0.18 per cent	(high carbohydrate (diet
Snyder, Pierce	1935	12M	24	0.061 per cent	Expressed as 61 mg. per 100 gm. liver
Blatherwick, Bradshaw, Ewing, Larson, Sawyer	1935	15M	27	0.555 per cent	Expressed as glucose
		14F	27	0.215 per cent	
Deuel, Hallman, Murray, Samuels	1937	15F	48	0.19 per cent	
		15M		0.59 per cent	
MacKay, Carne, Wick	1940	6M	24	0.18 per cent	
		12M	48	0.09 per cent	
		12F	48	0.14 per cent	
Nutter	1941	3F	24	0.03 per cent	

TABLE 25. AVERAGE CONCENTRATION OF GLYCOGEN IN LIVERS OF RATS FED GLUCOSE

Diet	Reproductive status	Number of animals	Age	Body wt.	Wt. of liver	Wt. of glycogen in liver	Per cent of glycogen in liver
			days	gm.	gm.	mg.	
Steenbock V	Virgin	8	151.9	176.1	5.3796	57.41	1.06
	Pregnant	8	135.2	251.2	6.7845	45.26	0.66
Pork I	Virgin	8	142.2	172.2	5.3408	80.70	1.51
	Pregnant	8	133.2	242.9	7.2914	46.84	0.64
Pork I Series IV	Pregnant	5	119.2	244.2	6.9876	53.39	0.76

TABLE 26. CONCENTRATION OF GLYCOGEN IN LIVERS OF RATS FED GLUCOSE

AS REPORTED BY OTHER INVESTIGATORS

Investigators	Date	Number of rats	Quantity of glucose administered	Absorption period	Concentration of liver glycogen	Remarks
				hr.		
Miller, Lewis	1932	8	725-890 mg.	3	1.97 per cent	
		11	177-266 mg.	3	0.63 per cent	
Cori	1926b	5-6M		1	0.38	0.183 (mg.
				2	0.91	0.188 (glucose
				3	2.66	0.176 (absorbed
				4	3.68	0.176 (per 100
				5	3.65	0.175 (gm. of (rat per (hr.
Cori, Cori	1926a	11	750 mg. absorbed	4	0.118 gm. per 100 gm. of body wt.	
MacKay, Wiok, Caine	1940	5F	1 cc. per sq. dm. of body	6	2.00 per cent	Glucose solution was 1 molar
Deuel, Hallman, Murray, Samuels	1937	10	600 mg. per 100 gm. body wt.	2	1.32 per cent	Additional doses of 500 mg. glucose per 100 gm. of body wt. were given on 3rd, 4th and 7th hrs.
		10		4	2.44 per cent	
		9		6	3.30 per cent	
		10		8	4.78 per cent	

TABLE 26 (cont.). CONCENTRATION OF GLYCOGEN IN LIVERS OF RATS FED GLUCOSE AS REPORTED BY OTHER INVESTIGATORS

Investigators	Date	Number of rats	Quantity of glucose administered	Absorption period	Concentration of liver glycogen	Remarks
				hr.		
Feyder, Pierce	1935	29	743-954 mg.	3	1557.0 mg. per 100 gm. liver 43.9 gm. per 100 gm. rat	An av. of 152 mg. was unabsorbed after 3 hrs.
Nutter, Murlin	1941	13F	824.9	3	1855.9 mg. per 100 gm. liver	Fasted, fatigued before feeding

glycogen obtained from the pregnant animals of the two groups from Series III. However, the pork-fed animals from Series IV that were killed on the twenty-second day of pregnancy had a slightly higher than normal concentration of glycogen in the liver. The difference, however, does not appear to be significant.

In both groups the virgin animals had smaller livers and a higher absolute and relative quantity of glycogen, than did the pregnant animals. These relations did not hold in all respects for the observations previously reported in Series I. However, in both series the difference between the percentage of glycogen in the pork-fed pregnant animals and the pork-fed virgins was greater than the difference between the control pregnant rats and control virgins. This discrepancy was not due to the post-starvation diet since it occurred with the glucose as well as with the test diets.

To summarize: there is a greater deposition of glycogen in the liver of the virgin rat fed the pork diet than in the virgin rat fed the stock diet, and this difference is not due to the quality of the post-starvation food. All data reported in the present study show that pregnancy is associated with a decreased percentage composition of liver glycogen. Results of the investigations with rats of Series III confirm the data

presented heretofore; that pregnancy in pork-fed rats reduces the relative amounts of glycogen in the liver more than it does in rats fed the Steenbock ration.

The results obtained in the present series (III and IV) were disappointing because the concentrations of glycogen in the liver of the pregnant animals were almost at the starvation level and, therefore, differences did not stand out. The four-hour absorption period chosen may be a factor. Although Cori (1926) states that the peak of glycogen formation occurs four hours after feeding glucose, it is possible that with the animals used in the present study and with the different physiological conditions imposed, the peak was reached somewhat earlier. It would be interesting to extend this study by feeding glucose to similar groups of animals and killing them at hourly intervals.

Also, perhaps, the effect of pregnancy should have been taken into consideration not only in setting the length of the absorption period but in calculating the weight of glucose to be administered. Larger quantities of glucose probably should have been given. The average weight of glucose received by the rat in the present study was 778 mg. However, this amount corresponds with that fed by Miller and Lewis, by Cori, and by Feyder and Pierce to non-gravid rats (Table 26). Miller and Lewis

observed an average of 1.97 per cent of liver glycogen three hours after feeding, and Feyder and Pierce an average of 1.56 per cent after three hours. These values are not so divergent from the 1.51 per cent found in the virgin pork-fed rats in the investigation here reported. In any further study with pregnant animals, it is therefore advised that a larger volume of 50 per cent glucose be administered, and that storage be studied at short intervals after feeding.

Glycogen Index

Although we attempted to administer the same amount of glucose to each rat, differences in activity of the animal and in technique caused fairly wide variations in the weight of glucose actually received by each rat. Also, it was possible that variations in absorption occurred in individual animals. Therefore, the "index of glycogen formation" used by Nutter and Murlin (1941) was adopted and the data for Series III and IV recalculated on this basis. The glycogen index may be expressed as the ratio:

$$\frac{\text{glycogen (mg. per 100 gm. of hepatic tissue)}}{\text{mg. sugar absorbed per 100 gm. of body wt.}}$$

The analytical data pertaining to the quantity of glucose administered to each rat are presented in Tables

XXXVII, XXXVIII, XXXIX, XL, and XLI (Appendix). A surprising variation occurred in the quantity of glucose contained in the washings from the catheter, although the amount of glucose originally contained in the syringe was very uniform. Probably the depth to which the tube was inserted in the rat, the way in which it was compressed in the oesophagus, and the convulsive movements of the animal during feeding resulted in the variations observed.

The quantity of glucose actually absorbed was next calculated for each animal by subtracting the average content of the starved intestinal tract for the corresponding group, from the weight of reducing substance actually found in the tract of each animal fed glucose. The quantities of glucose absorbed by each rat are reported in Tables XLII, XLIII, XLIV, XLV, and XLVI (Appendix). It will be noted that in many cases, less reducing material was present in the intestines than had previously been found for the starved rat. Therefore, some of the percentages representing glucose absorbed are greater than 100. Obviously, glucose absorption was complete in all groups.

The glycogen deposition in terms of the glycogen index for each animal is shown in Tables XLVII, XLVIII, XLIX, L, and LI (Appendix). Means for glucose adminis-

tered and absorbed, and for the glycogen indices are summarized in Table 27.

The glycogen indices also show that pregnancy reduces stores of glycogen. This analysis demonstrates no difference between the pregnant experimental and control rats, probably because the peak of glycogen analysis had been passed.

The index is higher in rats studied on the twenty-second day of pregnancy because they actually received more glucose per unit of body weight than did the rats of the other groups, due to their smaller size.

CONCENTRATION OF GLYCOGEN IN LIVER - SERIES V

An attempt was made to analyze the quantity of glycogen stored in livers of rats that were exhibiting toxic symptoms. Various difficulties presented themselves. Sometimes the animals died before the process of analysis could be initiated. Frequently the deaths occurred when no one was in the laboratory. Four animals were finally obtained, but since the treatment in respect to feeding and the symptoms exhibited varied so much, the results cannot be pooled. Therefore, each animal will be discussed separately. The weights, ages, liver weights and results of glycogen determinations are presented in

TABLE 27. AVERAGE ABSORPTION OF GLUCOSE AND GLYCOGEN INDICES FOR RATS OF SERIES III AND IV

Diet	Reproductive status	Number of rats	Wt. of glucose administered	Wt. of glucose absorbed	Per cent of glucose absorbed	Glycogen index
			mg.	mg.		
Pork I	Virgin	8	810.48	809.43	99.89	3.20
	Pregnant	8	792.73	793.77	100.14	1.97
Steenbock V	Virgin	8	767.72	767.65	100.02	2.41
	Pregnant	8	738.55	739.82	100.18	1.86
Pork I Series IV	Pregnant	5	778.39	777.89	99.94	2.43

Table XII.

Rat No. 28701

This rat was reared on Pork I and partially nephrectomized for the purpose of another study when 28 days old. On the twenty-second day of her first pregnancy she failed to give birth to her litter and showed bloody urine (one of the symptoms of toxemia). The food was removed in the evening and glucose was given at 7 a.m. on the morning of the twenty-third day and the liver when removed four hours later rated three plusses (+++) in respect to fat and friability. Nine resorptions were found in one horn of the uterus; one ovary had been accidentally removed at the time of the nephrectomy. The chemical analyses showed that 80.58 mg. of reducing material was present in the intestinal tract and that 827.12 mg. of glucose had been given so that 746.54 mg. was absorbed. The glycogen content of the liver was 0.84. The glycogen index was 1.63. This rat showed a definite abnormality in absorption and although the percentage concentration of glycogen in the liver was higher than the average for pork-fed pregnant rats, the glycogen index was somewhat lower. This was due to the fact that the body weight was only 145 gm.

There was little evidence that the animal described was typical of the animals showing acute toxemia, and the liver glycogen was apparently normal.

Rat Number 27984

Rat number 27984 was reared on Pork I and showed toxic symptoms on the morning of the twenty-third day of gestation. She was lethargic, cold to the touch, and had pale ears and paws. An average of 9 gm. of food had been consumed on the two preceding days and there was food in her cage when she was killed at 10 a.m. The stomach was full showing that food had been eaten. Upon autopsy the liver was only moderately fatty, but the kidneys were hemorrhagic and gorged with blood. There were seven dead feti, all very pale. No glycogen was found in the liver. This animal seemed quite typical of the sick rats and should have corresponded to the pregnant pork-fed animals of Series I.

Rat Number 28709

This rat was reared on Pork I and had also been partially nephrectomized. The litter had not been born on the twenty-third day of the first pregnancy, she had several shaking spells, and a lump appeared in the region

of the incision. She was killed on the afternoon of the twenty-third day and the liver analyzed. There was one live fetus, one ovary was missing, and the lump was connective tissue and fat. There were no symptoms of a true toxemia and the liver glycogen, 2.14 per cent, corresponded favorably with the results for the pregnant pork-fed rats of Series I.

Rat Number 24399

This rat was reared on the pork diet supplemented with lipocaic for the purposes of another study. On the twenty-second day of the first pregnancy she exhibited all the typical symptoms of toxemia; low body temperature, poor muscle tone, pale ears and paws. She was killed before death occurred and the liver removed for analysis. 10 dead feti were found upon autopsy, the kidneys were hemorrhagic, and the blood watery. No glycogen was found in the liver.

Thus, of the few animals studied in Series V, two definitely exhibited toxic symptoms, and these two had no detectable amounts of glycogen in the liver. This was not due to lack of food, since both had food in the stomach. The two partially nephrectomized animals had abnormalities due to the results of nephrectomy, and did

not show the typical syndrome.

There is some evidence, then, of derangement of carbohydrate metabolism, in rats exhibiting acute toxic symptoms.

SUMMARY AND CONCLUSIONS

The study herein reported is part of a series of investigations in progress in the Foods and Nutrition Laboratory of Iowa State College relating to the effect of feeding a diet containing pork muscle upon the reproductive performance of the albino rat. The experimental diet contained dried, canned pork muscle as its source of protein. Previous studies have shown gestational failure and the frequent occurence of a so-called "toxemia" of pregnancy in female rats reared upon this diet. Histological examinations and chemical analyses have shown the presence of fatty livers in the pork-fed rats. It is believed that a reciprocal relationship exists between the amount of fat and glycogen in the liver. The present study was undertaken in an effort to test the hypothesis that a derangement of carbohydrate metabolism may occur in the rats fed the pork diet. Animals reared upon the stock ration, a whole grain - casein diet designated as Steenbock V, served as controls. The experimental diet was called Pork I. The concentration of glycogen present in maternal and fetal livers was used as an index of the status of carbohydrate metabolism.

In the first series of experiments, the content of

glycogen in the livers of pregnant control rats was compared with that of virgins of the same age reared on the same diet. Subsequent to a 13-hour fast, all the animals were fed 4 gm. of the diet upon which they had been maintained. When seven hours had elapsed, they were stunned by a blow on the head and the livers quickly removed for analysis. Pregnant animals were killed on the 21.5 day of gestation and virgins when they were approximately the same age as the gravid rats. The livers were divided into halves, and each half dropped into a tared tube of potassium hydroxide. The quantity of glycogen was determined by the Good-Kramer modification of the Pflüger method. The Shaffer-Somogyi procedure for the analysis of glucose was used, and the amount of glucose present calculated by means of a regression based upon analyses of glucose solutions of known concentrations.

Both the absolute and relative amounts of glycogen present in the livers of the pregnant control animals were significantly higher than those in the livers of the pregnant pork-fed group. The average percentage composition of glycogen was 3.18 for the controls, and 2.57 for the experimental rats.

In each group the relative concentration of glycogen was higher in the livers of virgins than in the livers of

pregnant animals (4.08 vs. 2.57 per cent respectively in pork-fed rats and 3.58 vs. 3.18 per cent in the control rats). In the virgins fed the Steenbock diet, the absolute amount of glycogen was 213.1 mg. compared with 246.2 mg. in the livers of the pregnant animals. In the pork-fed virgins it was 231.7 mg. and in the pork-fed pregnant rats, 193.2 mg. In both groups, the livers of virgin animals were lighter than those of the pregnant rats and the difference was highly significant. It appears, therefore, that the effect of pregnancy in rats is to lower the relative amount of glycogen present in the liver, and that this decrease is greater in the case of the animals fed the pork diet than in animals reared on the stock ration. Pregnancy also results in an increase in the size of the liver.

The pregnant rats reared on the pork ration were next sorted into two groups, composed of those with total resorptions and those with living feti. Comparisons were made of absolute and relative amounts of glycogen in the livers of these two groups to determine whether the lowering of glycogen already noted in pork-fed animals could be attributed to the frequent occurrence of total resorptions. The liver weights of the rats with living feti were significantly higher than those with resorptions,

and the absolute weight of glycogen present in the liver was also significantly higher. However, the average relative concentration of glycogen in the livers of the two groups did not differ significantly. The average percentage of liver glycogen in the animals with living feti was significantly lower than that in the control rats, i.e., 2.78 per cent vs. 3.18 per cent. The presence of total resorptions, therefore, accounts for only a part of the fall in glycogen concentration observed in the livers of the experimental animals maintained on the pork diet.

To rule out variations due to differences in body weight, the weight of glycogen present in the liver of each animal was calculated in terms of mg. of glycogen per 100 gm. of gravid female, and in terms of mg. of glycogen per 100 gm. of female minus the weight of the intact uterus. The average weight of glycogen per 100 gm. of female (93.9 mg.) was higher in the control group than in the experimental group (86.2 mg.) but the difference was not significant. When the weight of the uterus was subtracted from the weight of the gravid female, the quantity of glycogen calculated per 100 gm. of rat was significantly higher in the control group than in the experimental group (120.9 mg. vs. 103.5 mg.).

To test the possibility that the quality of the post-starvation meal was responsible for the difference observed in the content of glycogen in the livers of the two groups, a second series of animals reared on Pork I was fed 4 gm. of the Steenbock ration seven hours before analysis of the liver. The average weight of glycogen found was 201.8 mg., the average percentage of glycogen, 2.34. Neither of these values differs significantly from the corresponding values for the pregnant pork-fed rats of the first series. The quality of the test meal, therefore, did not influence glycogen storage in the livers of the pregnant experimental rats.

Fetal livers were removed from part of the pregnant rats belonging to the first two series, and determinations of glycogen made as before. The livers from each litter were pooled. The average weight of liver glycogen per litter from the pork-fed group was 95.5 mg. which was significantly lower than the mean value, 119.2 mg. from the control group. The relative amounts of glycogen in the fetal livers from the two groups (6.10 vs. 5.92 per cent) did not differ significantly, but the average weight of fetal livers per litter was significantly higher in the control rats (1.9994 gm.) than in the pork-fed rats (1.6505 gm.). Thus, it seems that mothers reared on the stock diet were able to produce relatively

more fetal hepatic tissue and to store more glycogen in the fetal livers than were the experimental rats.

The data concerning both maternal and fetal livers were next examined by calculating the weight of maternal glycogen plus fetal glycogen in terms of 100 gm. of maternal and fetal liver and in terms of 100 gm. of gravid female. The control group produced a significantly higher total weight of hepatic tissue than did the experimental animals, and also a significantly greater amount of glycogen whether expressed in terms of the total weight of liver tissue or in terms of the weight of the female.

To determine whether the differences in glycogen content of the livers of the pregnant control and pork-fed animals noted, were due to the number of feti present, females from the two groups were matched according to number of feti, and the relative concentration of glycogen examined. In almost all cases, the control rat contained a higher percentage of maternal liver glycogen than did the pork-fed rat with the corresponding number of feti. In the control group the percentage of fetal glycogen increased as the number of feti decreased, but the concentration of maternal glycogen remained fairly consistent. The evidence accumulated in this series,

therefore, seems consistent when examined from all viewpoints, and points to the conclusion that production of glycogen is definitely decreased in pregnancy by feeding the diet containing pork muscle.

In the third and fourth series of animals studied, the relative absorption of glucose was investigated by determining the amount of reducing material present in the gastrointestinal tracts of control and experimental rats, both virgin and pregnant. First, tracts were analyzed from the four groups immediately after a starvation period to discover the quantities of reducing substances normally present. At the same time determinations of glycogen in the liver were made. When the liver was removed, the gastrointestinal tracts were ligated at the ileo-cecal and cardiac junctions, removed, split, and the contents washed into a volumetric flask. After appropriate dilution, glucose was determined again, using the Shaffer-Somogyi procedure. There was no consistent variation in the contents of the tracts of virgin and pregnant animals, nor in those of control and experimental animals. The average weight of reducing material present in all groups was 12.29 mg. which corresponds with values reported by other investigators.

Next, the quantities of reducing substances present

in the tracts of similar groups of rats which had been starved for 13 hours and killed four hours after the administration of 2.5 cc. of 50 per cent glucose by stomach tube, were determined. Again, there was no consistent variation between virgin and pregnant rats nor between the experimental and control animals. The values obtained were very similar to those obtained from analyses of the tracts of the starved animals. It was concluded that absorption of glucose was complete after four hours and that there was no abnormality in intestinal absorption in the pork-fed rats.

The effectiveness of the starvation period in depleting the store of liver glycogen was determined by analyzing the liver from starved pregnant and virgin animals previously fed the control and experimental diets. The analytical methods employed in the first two series of experiments were again used. The absolute and relative amounts of glycogen in the liver of the starved virgins were higher than those of the starved pregnant animals, presumably because fetal requirements deplete maternal stores. The percentage composition of glycogen was 0.04 in the livers of pregnant animals from both groups, 0.14 in the livers of pork-fed virgins, and 0.19 in the livers of control virgins. These values are all very low and it was believed that the glycogen stores of

the livers were largely depleted during the 13-hour starvation period.

To rule out some of the variation that might have been introduced in Series I from differences in the quantity and quality of the food eaten after the starvation period, the animals of the third series were fed a specified quantity of glucose. Subsequent to the 13-hour fast, pregnant and virgin rats, reared on both the stock and experimental diets, were given 2.5 cc. of a 50 per cent glucose solution by means of a fine catheter inserted into the stomach. The sugar solution was delivered from a syringe attached to the catheter. The actual amount of glucose administered was determined by making analyses of a similar volume delivered into a volumetric flask. After a four-hour interval the animals were stunned, the livers removed, and glycogen determined.

No difference could be observed in the absolute and relative amount of glycogen found in the livers of the pregnant controls (45.3 mg. and 0.66 per cent) and in the livers of the pregnant experimental rats (46.8 mg. and 0.64 per cent). In both groups, the livers of the virgins were lighter than those of the pregnant animals, and contained a higher absolute and relative quantity of glycogen. The livers of the pork-fed virgins contained an average

of 80.7 mg. of glycogen and an average percentage composition of 1.51; the livers of the control virgins, 57.41 mg., and 1.06 per cent. In this series, as in the first, there was a greater quantity of glycogen in the liver of the virgin rats reared on the pork diet than in those of the virgin rats reared on the stock diet. Again, it may be concluded that pregnancy is associated with a decreased percentage of liver glycogen. Also, in the pork-fed rats there was a greater difference between the level of glycogen stored in the livers of virgin and pregnant animals than there was in the Steenbock-fed animals. This difference is not attributable to the quality or quantity of the test meal.

The amount of glucose which was not absorbed by the rats of each group was calculated by subtracting the weight of reducing material normally present in the gastrointestinal tract of the corresponding group, from the amount actually found at the time of the liver analysis. This value was subtracted from the weight of glucose administered, and the per cent of glucose which was absorbed, calculated. The pregnant control rats absorbed an average of 100.18 per cent; the virgin control rats 100.02 per cent; the pork-fed pregnant animals, 100.14 per cent; and the pork-fed virgins, 99.89 per cent. It was again concluded that absorption

was complete after the four-hour interval.

A glycogen index was calculated for each rat in the third series by using the formula:

$$\frac{\text{glycogen (mg. per 100 gm. of hepatic tissue)}}{\text{mg. sugar absorbed per 100 gm. of body wt.}}$$

Glycogen indices thus obtained again showed that pregnancy reduces the stores of glycogen and that no difference exists between experimental and control animals.

The fourth series consisted of pregnant pork-fed rats killed on the twenty-second day of gestation and treated similarly to the pregnant pork-fed rats of the first series. No significant differences were noted in the two groups. It was concluded that no further breakdown of carbohydrate metabolism occurred on the last day of gestation in the experimental rats, and that if it does exist it occurs very suddenly and quickly when pregnancy disease develops.

The results of the determinations for the third and fourth series yielded little information as to the status of carbohydrate metabolism in the pregnant pork-fed animals because the values obtained for glycogen so nearly approached the starvation level. In any further investigation of this problem, it is recommended that larger quantities of glucose be administered and that the

absorption period be shortened, especially in working with pregnant animals.

The two animals of the fifth series that exhibited typical "toxic" symptoms showed no liver glycogen. It is believed that the derangement of carbohydrate metabolism noted in all pregnant rats reared on the pork diet on the twenty-first day of pregnancy becomes acute whenever an eclamptic condition develops.

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APPENDIX

PREPARATION AND STANDARDIZATION OF REAGENTS

CO_2-free Water

All solutions were made with CO_2-free water freshly prepared by boiling distilled water vigorously in a balloon flask for at least one-half hour. It was lightly stoppered, cooled, then stoppered tightly.

H_2SO_4, Approximately 1N

98 gm. or about 28 cc. of C.P. concentrated H_2SO_4 (sp. g. 1.84) was dissolved in distilled water and made up to 1 l. The normality was tested by titrating against standardized NaOH using methyl red as an indicator. It was never necessary to redilute the solution.

Some typical titrations are shown below:

TABLE I. STANDARDIZATION OF APPROXIMATELY 1N H_2SO_4

Solution tested	cc. H_2SO_4	cc. .9942N NaOH	Normality of H_2SO_4
I	10	10.05	0.9992
	10	10.06	
II	10	10.09	1.0036
	10	10.10	
II	15	15.12	1.0028
	15	15.12	

Sodium Hydroxide, Approximately 1N

A stock solution of saturated NaOH was made according to directions given by Hawk and Bergeim.[1] 110 gm. of high quality NaOH was shaken with 100 cc. of distilled water and allowed to stand covered until the Na_2CO_3 settled leaving a clear solution. Hawk and Bergeim indicate that a solution like this contains about 75 gm. of NaOH per 100 cc. A solution about 1.5N was made by diluting the saturated solution and titrating against carefully weighed portions of $KHC_8H_4O_4$ with phenolphthalein as the indicator. The exact normality was calculated and the solution rediluted to make it 1N. It was again standardized by titrating against weighed portions of $KHC_8H_4O_4$.

Some sample titrations are shown in Table II.

TABLE II. STANDARDIZATION OF NaOH

Solution tested	Wt. of $KHC_8H_4O_4$	Quantity of NaOH involved in titration	Concentration of NaOH	Normality of NaOH
		cc.	gm. per cc.	
I	.3892	1.21	.06304	1.576
	.5093	1.54	.06481	1.620
	.5776	1.78	.06359	1.589
				Av. 1.595
II	.5494	2.70	.03987	.9967
	.6025	2.96	.03989	.9972
	.5153	2.54	.03976	.9940
				Av. .9959

1. Hawk, P. B., and Bergeim, Olaf. 1937. Practical physiological chemistry, P. Blakiston's Son and Co., Inc., Philadelphia, ed. 11, p. 931.

30 Per Cent KOH

538 gm. of KOH was dissolved in 500 cc. of distilled water and heated. A 3 per cent $Ba(OH)_2$ solution was added until no more $BaCO_3$ precipitated. The solution was allowed to stand until clear, then filtered through washed asbestos. One cc. of the clear solution was diluted to 100 cc. with CO_2-free water and titrated against standardized HCl. The concentration was then calculated and a 30 per cent solution made by diluting the concentrated KOH.

Starch Indicator

2 gm. of soluble starch (Merck's Lintner) and 10 mg. of HgI_2 were triturated with a little water and the suspension added slowly to 1 l. of boiling water. The boiling was continued until the solution was clear. It was cooled, transferred to a glass stoppered bottle and stored in the refrigerator. This procedure was found superior to the method recommended by Hawk and Bergeim.[1]

Phenol Red

.04 gm. of phenol red was dissolved in 100 cc. of

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1. Hawk, P. B., and Bergeim, Olaf. 1937. Practical physiological chemistry, P. Blakiston's Son and Co., Inc., Philadelphia, ed. 11, p. 932.

distilled water. The solution was filtered and stored in the refrigerator to prevent the growth of mold.

Potassium Iodide and Potassium Oxalate

A 2.5 per cent solution of KI was prepared by weighing 2.5 gm. of crystalline KI on the torsion balance, dissolving in a little distilled water in a 100 cc. volumetric flask, diluting to the mark and mixing.

The solution containing 2.5 per cent each of KI and $K_2C_2O_4$ was made in similar manner. This solution was stored in a brown bottle and kept for about a week.

Copper-Iodometric Reagent 50

The copper-iodometric reagent was made up according to directions given by Shaffer and Somogyi (1933).

	gm. per l.
Na_2CO_3 (anhydrous).....	25.0
$NaHCO_3$.....	20.0
Rochelle salt.....	25.0
$CuSO_4 \cdot 5H_2O$.....	7.5
KIO_3, 0.1N as to I_2, cc. ...	200.0
KI.....	1.0

To retain all CO_2, the solution was made up as follows: The Na_2CO_3 and Rochelle salt were dissolved in about 500 cc. of distilled water. The $CuSO_4$ solution

(75 cc., 10 per cent) was added by pipette extending well below the surface of the liquid, the solution being stirred. The dry $NaHCO_3$ was next added. The solution was stirred to dissolve the bicarbonate. Then the KI was introduced. The solution was rinsed into a liter volumetric flask, the KIO_3 added, and the whole diluted to the mark and mixed. The solution was filtered through washed filter paper and stored in a brown Pyrex bottle.

The reagent was made up in quantities to last at least a year. Shaffer and Somogyi (1933) claim that the solutions remain unchanged for a year or more if kept in stoppered bottles protected from the light.

Standard Iodate Solutions

Solution A

3.567 gm. of pure KIO_3 was dissolved in 1 l. When treated with an excess of KI and H_2SO_4, this solution was 0.1N with respect to I_2. The solution was used to standardize the 0.1N $Na_2S_2O_3$ and was also used as a constituent of the copper reagent.

Solution B

A KIO_3 solution equivalent to 0.01N I_2 was prepared when needed, by dilution of Solution A. Usually 10 cc.

of Solution A was measured accurately into a 100 cc. volumetric flask, diluted to the mark and mixed. This solution was used for the standardization of the .005N $Na_2S_2O_3$.

$Na_2S_2O_3$

An approximately 0.1N stock solution was prepared by dissolving 24.83 gm. of $Na_2S_2O_3$ per l. About 10 cc. of 0.1N NaOH was added to increase its stability. The 0.1N stock solution of thiosulfate was standardized once or twice a year by titration with the .1N KIO_3 solution. About 50 cc. of water was added to 25 cc. of 0.1N KIO_3, 1 gm. of KI (iodate-free and weighed on a torsion balance), and 5 cc. of 1N H_2SO_4. $Na_2S_2O_4$ was added from a 50 cc. burette (B.S.) until the solution became straw colored. One cc. of the starch indicator was added and the titration continued until the blue color of the starch and iodine just disappeared.

Some typical standardizations are shown in Table III. The stability of 0.1N $Na_2S_2O_3$ is shown by Table IV.

From the normality factor of the 0.1N $Na_2S_2O_3$ solution the amount required to give 1 l. of .005N solution was calculated. This solution was prepared about once a week since Shaffer and Somogyi (1933) state that this

TABLE III. STANDARDIZATION OF APPROXIMATELY 0.1N $Na_2S_2O_3$

$Na_2S_2O_3$ solution tested	KIO_3 solution used	Normality of KIO_3	$Na_2S_2O_3$ used	Normality of $Na_2S_2O_3$
			cc.	
1	1	0.1000	24.91 24.94	0.10032
1	2	0.1000	24.95 24.90	0.10032
1	3	0.9920	24.61 24.62	0.10073
				Av. 0.10045
2	1	0.1000	24.78 24.81	0.10082
2	2	0.1000	24.78 24.78	0.10082
2	3	0.1000	24.80 24.80	0.10080
				Av. 0.10083
3	1	0.1000	24.60 24.58	0.10162
4	1	0.1000	24.78 24.75	0.10097
5	1	0.1000	24.91 24.90	0.10040

TABLE IV. STANDARDIZATION OF

0.10083N $Na_2S_2O_3$ SOLUTION NO. 2

AFTER AN INTERVAL OF TWO MONTHS

KIO_3 solution used	Normality of KIO_3	$Na_2S_2O_3$ used	Normality of $Na_2S_2O_3$
		cc.	
1	0.1000	24.77 24.75	0.10097
2	0.1000	24.70 24.78	0.10105
3	0.1000	24.78 24.80	0.10085
			Av. 0.10095

solution when alkaline retains its titer for some days. The dilute solution was standardized by titrating with .01N KIO_3. 10 cc. of the .01N KIO_3 was measured into a flask with 1 cc. of N H_2SO_4 and 2 cc. of 2.5 per cent KI and titrated with the approximately .005N thiosulfate from a 50 cc. burette (B.S.) using starch as an indicator as before.

Some standardizations are shown in Table V.

TABLE V. STANDARDIZATION OF APPROXIMATELY .005N $Na_2S_2O_3$

$Na_2S_2O_3$ solution tested	KIO_3 solution used	Normality of KIO_3	$Na_2S_2O_3$ used	Normality of $Na_2S_2O_3$
			cc.	
1	1	.0100	18.34 18.32	.005455
1	2	.0992	18.00 18.05	.005505
				Av. .005485
2	1	.0100	19.66 19.62	.005092
2	2	.0100	19.50 19.50	.005128
2	3	.0992	19.47 19.40	.005144
				Av. .005121
3	1	.0100	19.79 19.84	.005045
3	2	.0100	19.90 19.84	.005033
3	3	.0100	19.82 19.90	.005035
				Av. .005023
4	1	.0100	19.50 19.42	.005139
4	2	.0100	19.48 19.57	.005123
4	3	.0100	19.52 19.51	.005123
				Av. .005128

TABLE VI. INDIVIDUAL NUMBERS OF RATS USED IN EXPERIMENTAL AND CONTROL GROUPS OF SERIES I AND SERIES II

Series I				Series II
Steenbock V		Pork I		Pork I
Pregnant	Virgin	Pregnant	Virgin	Pregnant
21810	25533	21813	27756	25387
22524	25424	21909	27804	25421
21910	25758	21825	27918	25529
22574	27755	21826	28050	25644
23388	27919	22240	28106	25754
22615	28051	22238	28237	25796
22576	28107	22239	28322	25848
22523	28238	22160	28335	25917
21970		22164		26115
21893		21773		26208
22039		21812		
21985		21982		
21996		21892		
24364		21971		
24419		22241		
24494		24051		
24558		24625		
24631		24652		
24436		24672		
24653		24685		
24623		24632		
24531		23934		
24686		24729		
		24607		
		24660		
		24085		
Total 23	8	26	8	10

TABLE VII. INDIVIDUAL NUMBERS OF RATS USED IN EXPERIMENTAL AND CONTROL GROUPS OF SERIES III, SERIES IV AND SERIES V

Series III								Series IV	Series V
Starved groups				Glucose-fed groups				Glucose-fed	Sick
Steenbock V		Pork I		Steenbock V		Pork I		Pork I	Pork I
Pregnant	Virgin	Pregnant	Virgin	Pregnant	Virgin	Pregnant	Virgin	Pregnant	Pregnant
25416	25417	25388	25389	25920	25921	25849	25850	28412	28701
25646	25648	25420	25422	26080	26081	25918	25919	28575	27984
25967	25968	25530	25531	26199	26200	25797	25798	28798	28709
25851	25852	25645	25647	26270	26271	26116	26117	28788	24399
25799	25800	25755	25756	26276	27929	26209	26210	28893	
				27927	28336	28290	28292		
				28115	28117	28114	28116		
				28291	28293	28489	27928		
Total 5	5	5	5	8	8	8	8	5	4

TABLE VIII. STANDARDIZATION OF COPPER REAGENT NO. 1

Glucose solution tested	Glucose per liter	Glucose per 5 cc.	.005N $Na_2S_2O_3$ used	Glucose solution tested	Glucose per liter	Glucose per 5 cc.	.005N $Na_2S_2O_3$ used
	gm.	mg.	cc.		gm.	mg.	cc.
1	0.1608	0.8040	7.26	8	0.2422	1.2110	11.09
			7.31				11.06
2	0.2017	1.0085	9.06				10.27
			9.12				10.30
			8.33	9	0.2828	1.4140	12.31
			9.08				13.01
			8.69				13.00
3	0.2429	1.2145	11.09	10	0.3015	1.5075	14.02
			11.09				14.94
4	0.2816	1.4080	12.65				14.91
			12.74				14.15
5	0.3006	1.5030	13.64	11	0.1596	0.7980	7.25
			13.60				7.21
			13.90	12	0.2038	1.0190	9.20
			13.95				9.17
6	0.1597	0.7985	6.71	13	0.2426	1.2130	10.66
			6.74				10.65
			6.76	14	0.2826	1.4130	12.82
			6.50				12.81
			6.18	15	0.3016	1.5080	13.68
7	0.2023	1.0115	8.41	16	0.2426	0.7278*	6.10
			8.38				6.00
			8.53				5.99
			8.57	17	0.3006	0.9018*	8.08
							7.95

* 3 cc. aliquots.

TABLE IX. STANDARDIZATION OF COPPER REAGENTS NO. 3 AND 4.

Glucose solution tested	Glucose per liter	Glucose per 5 cc.	.005N $Na_2S_2O_3$ used for reagent no. 3	.005 N $Na_2S_2O_3$ used for reagent no. 4
	gm.	mg.	cc.	cc.
1	0.2402	1.20	10.49	10.69
2	0.2868	1.43	12.78	12.83
3	0.1756	0.88	7.49	7.73
4	0.2067	1.03	9.11	9.18

TABLE X. CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT CONTROL RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti+ placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
21810	245	135	53.8	46.1	4.2209 3.1242	7.3451	132.82 118.30	3.15 3.79	251.12	3.42
22524	264	141	68.0	66.2	4.3392 3.6536	7.9927	150.30 135.60	3.46 3.71	285.90	3.58
21910	230	142	44.5	39.0	3.8410 3.2356	7.0766	142.03 122.45	3.70 3.78	264.48	3.74
22574	248	136	48.0	40.8	4.3519 3.8175	8.1694	108.29 111.96	2.49 2.93	220.25	2.70
23388	286	205	68.0	52.0	3.7270 4.6714	8.3984	131.36 155.92	3.52 3.34	287.28	3.42
22615	225	131	53.5	46.5	3.3354 3.4629	6.7983	113.25 80.79	3.40 2.33	194.04	2.86
22576	244	129	52.7	47.7	3.7274 3.8767	7.6041	145.62 149.85	3.91 3.86	295.47	3.88
22523	248	149	55.5	49.0	3.2635 3.7351	6.9986	118.67 131.27	3.64 3.51	249.94	3.57
21970	278	150	63.0	57.0	3.8604 4.3101	8.1705	109.66 113.81	2.84 2.64	223.47	2.74

TABLE X (cont.). CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT CONTROL RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
21893	228	132	50.2	45.2	3.2368 3.5485	6.7853	57.25 116.80	1.78* 3.29	223.25	3.29
22039	249	114	58.3	52.7	3.5257 3.5022	7.0279	85.02 86.22	2.41 2.46	171.24	2.44
21985	290	162	68.0	61.5	3.8949 3.9716	7.8665	93.66 93.11	2.40 2.34	186.77	2.37
21996	265	156	58.5	51.5	4.0122 3.6373	7.6495	104.97 99.00	2.62 2.72	203.97	2.67
24364	283	147	66.0	59.6	4.5447 4.6208	9.1655	156.93 159.31	3.45 3.45	316.24	3.45
24419	248	146	--	--	3.2763 3.5471	6.8234	103.96 136.39	3.17 3.84	240.35	3.52
24494	272	146	50.0	43.5	3.9263 4.5502	8.4765	138.82 152.70	3.54 3.36	291.52	3.44
24558	292	144	74.5	67.5	3.7844 5.3732	9.1576	122.99 152.06	3.25 2.83	275.05	3.00

* Omitted from average. Total wt. of glycogen estimated.

TABLE X (cont.). CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT CONTROL RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
24631	271	139	74.6	68.6	4.1194 3.6341	7.7535	133.21 119.78	3.23 3.30	252.99	3.26
24436	280	168	64.0	56.7	3.5333 4.1268	7.6601	110.03 126.41	3.11 3.06	236.44	3.09
24653	285	141	63.3	55.8	3.5859 4.5156	8.1014	87.96 101.48	2.45 2.45	189.44	2.34
24623	264	144	52.8	46.0	3.4840 4.5442	8.0282	108.75 124.75	3.12 2.74	233.50	2.91
24531	275	168	62.0	55.5	3.9888 4.7174	8.7062	140.47 156.93	3.52 3.33	297.40	3.42
24666	248	155	41.5	37.0	2.8910 3.4525	6.3435	103.04 146.45	3.56 4.24	249.49	3.93
Total	6018	3380				178.0988			5639.60	73.04
Av.	261.7	146.9				7.7434			245.20	3.18

TABLE XI. CONCENTRATION OF LIVER GLYCOGEN IN VIRGIN CONTROL RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	mg.		mg.	
25533	177	134	2.6455 3.2733	5.9198	93.88 111.87	3.55 3.42	205.72	3.48
25424	198	171	3.0503 3.0627	6.1130	96.06 94.40	3.15 3.08	190.46	3.12
25758	180	133	3.6965 2.7276	6.4241	121.34 99.36	3.28 3.64	220.70	3.43
27755	163	161	2.3366 2.6069	4.9435	100.28 111.87	4.29 4.29	212.15	4.29
27919	154	158	2.9614 2.6481	5.6095	109.12 100.75	3.68 3.80	209.87	3.74
28051	191	150	3.1432 2.8498	5.9930	96.70 91.73	3.08 3.22	188.43	3.14
28107	184	143	2.9548 3.1577	6.1125	113.53 124.01	3.84 3.93	237.54	3.89
28238	184	146	3.5379 3.2322	6.7701	123.55 116.47	3.49 3.60	240.02	3.54
Total	1431	1196		47.8855			1704.89	28.63
Av.	178.8	149.50		5.9856			213.11	3.58

TABLE XII. CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT PORK-FED RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
21813	236	126	25.6	--	4.2374 4.4563	8.6937	113.72 118.44	2.68 2.66	232.16	2.67
21909	250	111	--	--	3.4784 4.3659	7.8443	82.08 98.45	2.36 2.25	180.52	2.30
21825	260	127	42.0	36.0	4.0665 4.1959	8.2624	91.18 87.60	2.24 2.09	178.78	2.16
21826	266	144	48.1	42.8	4.3304 4.1399	8.4703	140.56 138.54	3.24 3.35	279.10	3.30
22240	236	141	26.5	20.0	3.8532 3.8001	7.6533	113.81 113.98	2.95 3.00	227.79	2.98
22238	265	115	56.5	49.5	4.0174 4.1431	8.1605	127.42 126.31	3.17 3.05	253.73	3.11
22239	240	112	45.8	39.3	3.4639 4.4459	7.9098	114.17 132.93	3.30 2.99	247.10	3.12
22160	232	114	54.0	47.5	4.2164 2.9935	7.2099	120.43 93.02	2.86 3.11	213.45	2.96
22164	249	125	59.0	52.0	3.8420 3.9144	7.7564	63.87 64.51	1.66 1.65	128.38	1.66

TABLE XII (cont.). CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT PORK-FED RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
21773	250	149	40.8	34.2	3.6757 3.7016	7.3773	58.63 75.55	1.60* 2.04	150.50	2.04
21812	248	158	39.3	34.2	3.9564 3.7728	7.7292	108.75 105.07	2.75 2.78	213.82	2.77
21982	242	121	10.7	--	3.1518 4.0010	7.1528	61.75 75.09	1.95 1.88	136.66	1.91
21892	204	170	25.0	21.3	3.3110 2.9888	6.2998	136.79 120.97	4.13 4.05	257.76	4.09
21971	300	152	58.5	49.5	4.1213 4.7538	8.8751	84.84 91.74	2.06 1.93	176.58	1.99
22241	207	126	--	--	4.0674 3.9262	7.9936	98.63 128.06	2.42 3.26	226.69	2.84
24051	240	185	--	--	4.5566 4.2923	6.8489	73.53 78.48	1.61 1.83	152.01	1.72
24625	186	126	--	--	3.4668 2.5867	6.0535	74.44 56.97	2.15 2.20	131.41	2.17
24652	238	122	50.0	45.5	4.1814 3.2179	7.3993	139.92 113.53	3.35 3.53	253.45	3.42

TABLE XII (cont.). CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT PORK-FED RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
24672	255	123	40.0	34.0	3.8608 3.7876	7.6484	111.68 113.72	2.89 3.00	225.40	2.95
24685	217	126	--	--	2.6387 3.5579	6.2266	57.34 71.96	2.17 2.00	129.30	2.08
24632	214	136	--	--	3.3617 3.2614	6.6231	86.03 85.48	2.56 2.62	171.51	2.59
23934	226	222	--	--	3.7206 3.2383	6.9589	71.14 64.33	1.91 1.99	135.47	1.95
24729	205	120	--	--	2.8324 3.6434	6.4758	93.02 110.03	3.28 3.02	205.05	3.14
24607	231	153	--	--	4.3126 3.5975	7.9101	113.07 102.13	2.62 2.84	215.20	2.72
24660	203	163	--	--	3.0132 3.6759	6.6891	48.06 34.72	1.60 0.94	82.78	1.24
24058	---	240	--	--	3.5300 3.8110	7.3410	108.20 113.53	3.06 2.98	221.73	3.02
Total	5900	3707				195.5631			5024.33	66.90
Av.	236.0	142.6				7.5216			193.24	2.57

* Boiled over during analysis. Omitted from average. Total wt. of glycogen estimated.

TABLE XIII. CONCENTRATION OF LIVER GLYCOGEN IN VIRGIN PORK-FED RATS OF SERIES I

Number of rat	Wt. of rat	Age of rat	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	mg.		mg.	
27756	177	161	2.6661 2.7562	5.4223	88.33 95.32	3.31 3.46	185.65	3.39
27804	146	171	2.0707 1.8153	3.8860	86.86 85.48	4.19 4.71	172.34	4.43
27918	178	161	2.8958 3.2497	6.1455	121.16 138.26	4.18 4.25	259.42	4.22
28050	174	150	2.4580 3.1884	5.6464	106.08 127.51	4.32 4.00	233.59	4.14
28106	173	143	3.0888 2.9679	6.0567	137.44 135.14	4.45 4.55	272.58	4.50
28237	175	146	3.3446 3.1854	6.5300	120.76 116.65	3.61 3.66	237.41	3.64
28322	172	143	2.5610 2.3898	4.9508	110.59 99.55	4.32 4.16	210.14	4.24
28335	195	142	3.3888 3.5723	6.9611	145.44 139.00	4.29 3.89	284.44	4.09
Total	1390	1217		45.5988			1853.57	32.65
Av.	173.7	152.12		5.6998			231.69	4.08

TABLE XIV. CONCENTRATION OF LIVER GLYCOGEN IN TERMS OF 100 GM. OF PREGNANT CONTROL RATS OF SERIES I

Number of rat	Wt. of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of glycogen in liver	Wt. of glycogen per 100 gm. of gravid female	Wt. of glycogen per 100 gm. of gravid female minus uterus
	gm.	gm.	gm.	mg.	mg.	mg.
21810	245	53.8	46.1	251.12	102.49	131.34
22524	264	68.0	66.2	285.90	108.30	145.87
21910	230	44.5	39.0	264.48	114.99	142.58
22574	248	48.0	40.8	220.26	88.81	110.12
23388	286	68.0	52.0	287.28	100.45	131.78
22615	225	53.5	46.5	194.04	86.24	113.14
22576	244	52.7	47.7	295.47	121.09	154.45
22523	248	55.5	49.0	249.94	100.78	129.84
21970	278	63.0	57.0	223.47	80.38	103.94
21893	228	50.2	45.2	223.25	97.92	125.56
22039	249	58.3	52.7	171.24	68.77	89.80
21985	290	68.0	61.5	186.77	64.40	84.13
21996	265	58.5	51.5	203.97	76.97	98.77
24364	283	66.0	59.6	316.24	111.74	145.73
24419	248	--	--	240.35	96.92	--
24494	272	50.0	43.5	291.52	107.18	131.32
24558	292	74.5	67.5	275.05	94.20	126.46
24631	271	74.6	68.6	252.99	93.35	128.81
24436	280	64.0	56.7	236.44	84.44	109.46
24653	285	63.3	55.8	189.44	66.47	85.45
24623	264	52.8	46.0	233.50	88.45	110.56
24531	275	62.0	55.5	297.40	108.14	139.62
24686	248	41.5	37.0	249.49	100.60	120.82
Total	6018	1290.7			2163.08	2659.55
Av.	261.7	58.7			94.05	120.89

TABLE XV. CONCENTRATION OF LIVER GLYCOGEN IN TERMS OF 100 GM. OF PREGNANT PORK-FED RATS OF SERIES I

Number of rat	Wt. of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of glycogen in liver	Wt. of glycogen per 100 gm. of gravid female	Wt. of glycogen per 100 gm. of gravid female minus uterus
	gm.	gm.	gm.	mg.	mg.	mg.
21813	238	25.6	--	232.16	98.37	110.34
21909	250	--	--	180.52	72.21	--
21825	260	42.0	36.0	178.78	68.76	82.01
21826	266	48.1	42.8	279.10	104.92	128.09
22240	236	26.5	20.0	227.79	96.52	108.73
22238	235	56.5	49.5	253.73	95.75	121.11
22239	240	45.8	39.3	247.10	102.96	127.24
22160	232	54.0	47.5	213.45	92.00	119.92
22164	249	59.0	52.0	128.38	51.56	65.17
21773	250	40.8	34.2	150.50	60.20	71.94
21812	248	39.3	34.2	213.82	86.22	102.45
21982	242	10.7	--	136.66	56.47	59.08
21892	204	25.0	21.3	257.76	126.35	144.00
21971	300	58.5	49.5	176.58	58.86	73.12
22241	207	--	--	226.69	109.51	--
24051	240	--	--	152.01	63.34	--
24625	186	--	--	131.41	70.65	--
24652	238	50.0	45.5	253.45	106.49	134.81
24672	255	40.0	34.0	225.40	88.39	104.84
24685	217	--	--	129.30	59.58	--
24632	214	--	--	171.51	80.14	--
23934	226	--	--	135.47	59.94	--
24729	205	--	--	203.05	99.05	--
24607	231	--	--	215.20	93.16	--
24660	203	--	--	82.78	40.78	--
24058	---	--	--	221.73	--	--
Total	5900				2042.18	1552.85
Av.	3				81.69	103.52

Av. only for rats with living feti -- 248.1

TABLE XVI. CONCENTRATION OF LIVER GLYCOGEN IN PORK-FED RATS SHOWING TOTAL RESORPTIONS AND IN PORK-FED RATS WITH LIVING FETI

Rats with total resorptions				Rats with living feti			
Number of rat	Wt. of liver	Wt. of glycogen in liver	Per cent glycogen in liver	Number of rat	Wt. of liver	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	mg.			gm.	mg.	
22241	7.9936	226.69	2.84	21909	7.8443	180.52	2.30
24625	6.0535	131.41	2.17	21825	8.2624	178.78	2.16
24685	6.2266	129.30	2.08	21773	7.3773	150.50	2.04
24632	6.6231	171.51	2.59	21826	8.4703	279.10	3.30
23934	6.9589	135.47	1.95	22160	7.2099	213.45	2.96
24729	6.4758	203.05	3.14	21812	7.7292	213.82	2.77
24607	7.9101	215.20	2.72	22239	7.9098	247.10	3.12
24058	7.3410	221.73	3.02	22164	7.7564	128.38	1.66
21982	7.1528	136.66	1.91	22238	8.1605	253.73	3.11
24051	8.8489	152.01	1.72	21971	8.8751	176.58	1.99
24660	6.6891	82.78	1.24	21892	6.2998	257.76	4.09
				22240	7.6553	227.79	2.98
				24652	7.3993	253.45	3.42
				24672	7.6484	225.40	2.95
Total	78.2734	1805.81	25.38		108.5960	2986.36	38.85
Av.	7.1158	164.16	2.31		7.7568	213.31	2.78

TABLE XVII. CONCENTRATION OF LIVER GLYCOGEN IN RATS OF SERIES II

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
25387	262	129	49.0	42.7	4.0687 5.0542	9.1229	103.14 118.86	2.53 2.35	222.00	2.43
25421	268	116	61.5	55.1	4.4446 4.0350	8.4796	123.36 124.93	2.78 3.10	248.29	2.93
25529	276	111	64.8	57.8	4.8318 4.5805	9.4123	62.40 52.75	1.29 1.15	115.15	1.22
25644	246	116	64.2	58.0	4.4554 3.6326	8.0880	90.35 53.85	2.03 1.48	144.20	1.78
25754	248	117	57.2	50.7	4.9923 2.4585	7.4508	126.03 99.55	2.52 4.05*	187.76	2.52
25796	228	113	53.5	48.0	3.8129 4.0754	7.8883	94.77 97.53	2.48 2.39	192.30	2.43
25848	256	117	46.4	40.0	5.2339 5.0416	10.2755	126.77 128.33	2.42 2.54	255.10	2.48
25917	254	130	50.5	43.3	4.2828 4.7606	9.0434	112.24 133.40	2.62 2.80	245.64	2.71
26115	239	113	60.0	53.0	3.4707 4.7371	8.2078	76.01 89.53	2.19 1.89	165.54	2.02

* Omitted from average. Total wt. of glycogen estimated.

TABLE XVII (cont.). CONCENTRATION OF LIVER GLYCOGEN IN RATS OF SERIES II

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
26208	237	112	59.0	53.9	3.9676 4.4309	8.3985	121.62 118.40	3.06 2.67	240.02	2.86
Total	2514	1174	566.1	502.5		86.3671			2016.00	23.38
Av.	251.4	117.4	56.6	50.2		8.6367			201.60	2.34

TABLE XVIII. CONCENTRATION OF FETAL GLYCOGEN FROM CONTROL RATS OF SERIES I

Number of rat	Wt. of feti plus placentae	Number of feti	Wt. of fetal livers	Wt. of glycogen	Per cent glycogen
	gm.		gm.	mg.	
23388	52.0	13	2.2504	126.59	5.62
24364	59.6	12	2.2271	118.58	5.32
24419	--	8	2.0481	141.24	6.90
24494	43.5	9	1.3010	114.08	8.77
24558	67.5	15	2.1796	109.66	5.03
24631	68.6	14	2.1191	114.58	5.41
24436	56.7	12	2.4543	128.06	5.22
24653	55.8	12	2.3924	140.84	5.89
24623	46.0	7	1.5530	103.60	6.67
24531	55.5	11	1.9377	121.62	6.28
24686	37.0	9	1.5309	92.19	6.02
Total	542.2	122	21.9936	1311.04	67.13
Av.	54.2	11.0	1.9994	119.18	6.10

TABLE XIX. CONCENTRATION OF FETAL GLYCOGEN FROM PORK-FED RATS

Number of rat	Wt. of feti plus placentae	Number of feti	Wt. of fetal livers	Wt. of glycogen	Per cent glycogen
	gm.		gm.	mg.	
25387	42.7	9	1.8187	106.45	5.85
25421	55.1	13	1.8890	100.11	5.30
25529	57.8	14	2.2041	107.64	4.88
25644	58.0	11	1.8575	122.17	6.58
25754	50.7	11	1.4356	94.87	6.61
25796	48.0	10	1.3898	90.99	6.55
25848	40.0	8	1.4633	95.04	6.50
25917	43.3	9	1.8033	93.57	5.19
26115	53.0	11	1.3837	88.06	6.36
26208	53.9	10	1.2026	78.31	6.51
24652	45.5	9	2.0030	73.62	3.68
24672	34.0	7	1.3557	95.68	7.06
Total	582.0	122	19.8063	1146.51	71.07
Av.	48.5	10.2	1.6505	95.54	5.92

TABLE XX. CONCENTRATION OF MATERNAL AND

No. of rat	No. of feti	Wt. of feti and placentae	Wt. of mother	Wt. of maternal liver	Wt. of fetal liver	Wt. of fetal and maternal liver	Wt. cog mat liv
		gm.	gm.	gm.	gm.	gm.	m
23388	13	52.0	286	8.3984	2.2504	10.6488	28
24364	12	59.6	283	9.1655	2.2271	11.3926	31
24419	8		248	6.8234	2.0481	8.8715	24
24494	9	43.5	272	8.4765	1.3010	9.7775	29
24558	15	67.5	292	9.1576	2.1796	11.3372	27
24631	14	68.6	271	7.7535	2.1191	9.8726	25
24436	12	56.7	280	7.6601	2.4543	10.1144	23
24653	12	55.8	285	8.1014	2.3924	10.4938	18
24623	7	46.0	264	8.0282	1.5530	9.5812	23
24531	11	55.5	275	8.7062	1.9377	10.6439	29
24686	9	37.0	248	6.3435	1.5309	7.8744	24
Total	122	342.2	3004	88.6143	21.9936	110.6079	286
Average	11.01	54.2	273.00	8.0558	1.9994	10.0553	26

MATERNAL AND FETAL LIVER GLYCOGEN IN CONTROL RATS

of fe- and ernal er	Wt. of gly- cogen in maternal liver	Wt. of glycogen in fetal liver	Fetal gly- cogen and maternal glycogen	Maternal + fetal glycogen per 100 gm. of fetal and maternal liver	Maternal + fetal glycogen per 100 gm. gravid female
m.	mg.	mg.	mg.	mg.	mg.
.6488	287.28	126.59	413.87	3886.54	144.71
.3926	316.24	118.58	434.82	3816.69	133.65
.8715	240.35	141.24	381.59	4301.30	153.87
.7775	291.52	114.08	405.60	4148.30	149.12
.3372	275.05	109.66	384.71	3393.34	137.50
.8726	252.99	114.58	367.57	3723.13	135.63
.1144	236.44	128.06	364.50	3603.77	130.18
.4938	189.44	140.84	330.28	3147.38	115.89
.5812	233.50	103.60	337.10	3518.34	127.69
.6439	297.40	121.62	419.02	3936.71	152.37
.8744	249.49	92.19	341.68	4339.12	137.77
.6079	2869.70	1311.04	4180.74	41814.62	1518.38
.0553	260.88	119.18	380.07	3801.33	138.03

TABLE XXI. CONCENTRATION OF MATERNA

No. of rat	No. of feti	Wt. of feti and placentae	Wt. of mother	Wt. of maternal liver	Wt. of fetal liver	Wt. of fetal and maternal liver	Wt oo ma li
		gm.	gm.	gm.	gm.	gm.	
25387	9	42.7	262	9.1229	1.8187	10.9416	22
25421	12	55.1	268	8.4796	1.8890	10.3686	24
25529	14	57.8	276	9.4122	2.2041	11.6164	11
25644	11	58.0	246	8.0880	1.8575	9.9455	14
25754	11	50.7	248	7.4508	1.4356	8.8864	22
25796	10	48.0	228	7.8882	1.3898	9.2781	19
25848	8	40.0	256	10.2755	1.4633	11.7388	25
25917	9	43.3	254	9.0434	1.8033	10.8467	24
26115	11	53.0	239	8.2078	1.3837	9.5915	16
26208	10	53.9	237	8.3985	1.2026	9.6011	24
24652	9	45.5	238	7.3993	2.0030	9.4023	25
24672	7	34.0	255	7.6484	1.3557	9.0041	22
Total	122	582.0	3007	101.4148	19.8063	121.2211	253
Average	10.2	48.5	250.58	8.4512	1.6505	10.1018	21

TRATION OF MATERNAL AND FETAL LIVER GLYCOGEN IN CONTROL RATS

f	Wt. of fetal and maternal liver	Wt. of glycogen in maternal liver	Wt. of glycogen in fetal liver	Fetal glycogen and maternal glycogen	Maternal + fetal glycogen per 100 gm. of fetal and maternal liver	Maternal + glycogen p gm. gravid
	gm.	mg.	mg.	mg.	mg.	mg.
	10.9416	222.00	106.45	328.45	3001.85	125.36
	10.3686	248.29	100.11	348.40	3360.14	130.00
	11.6164	115.15	107.64	222.79	1917.80	80.72
	9.9455	144.20	122.17	266.37	2678.29	108.28
	8.8864	225.58	94.87	320.45	3606.07	129.21
	9.2781	192.30	90.99	283.29	3053.31	124.47
	11.7388	255.10	95.04	350.14	2982.75	136.77
	10.8467	245.64	93.57	339.21	3127.31	133.55
	9.5915	165.54	88.06	253.60	2644.00	106.11
	9.6011	240.02	78.31	318.32	3315.56	134.32
	9.4023	253.45	73.62	327.07	3478.62	137.42
	9.0041	225.40	95.68	321.08	3565.93	125.91
	121.2211	2532.67	1146.51	3679.18	36731.63	1472.12
	10.1018	211.06	95.54	306.60	3060.97	122.68

'ERNAL AND FETAL LIVER GLYCOGEN IN CONTROL RATS

Wt. of glycogen in maternal liver	Wt. of glycogen in fetal liver	Fetal glycogen and maternal glycogen	Maternal + fetal glycogen per 100 gm. of fetal and maternal liver	Maternal + fetal glycogen per 100 gm. gravid female
mg.	mg.	mg.	mg.	mg.
222.00	106.45	328.45	3001.85	125.36
248.29	100.11	348.40	3360.14	130.00
115.15	107.64	222.79	1917.80	80.72
144.20	122.17	266.37	2678.29	108.28
225.58	94.87	320.45	3606.07	129.21
192.30	90.99	283.29	3053.31	124.47
255.10	95.04	350.14	2982.75	136.77
245.64	93.57	339.21	3127.31	133.55
165.54	88.06	253.60	2644.00	106.11
240.02	78.31	318.32	3315.56	134.32
253.45	73.62	327.07	3478.62	137.42
225.40	95.68	321.08	3565.93	125.91
2532.67	1146.51	3679.18	36731.63	1472.12
211.06	95.54	306.60	3060.97	122.68

TABLE XXII. CONCENTRATION OF LIVER GLYCOGEN IN CONTROL AND PORK-FED RATS MATCHED AS TO NUMBER OF FETI

Pork I rats					Steenbock V rats				
No. of rat	Wt. of feti + placentae	No. of feti	Per cent of fetal liver glycogen	Per cent of maternal liver glycogen	No. of rat	Wt. of feti + placentae	No. of feti	Per cent of fetal liver glycogen	Per cent of maternal liver glycogen
	gm.					gm.			
25529	57.8	14	4.88	1.22	24631	68.6	14	5.41	3.26
25421	55.1	13	5.30	2.93	23388	52.0	13	5.62	3.42
25644	58.0	11	6.58	1.78	24531	55.5	11	6.28	3.42
25754	50.7	11	6.61	2.52					
26115	53.0	11	6.36	2.02					
25917	43.3	9	5.19	2.71	24494	43.5	9	8.77	3.44
24652	45.5	9	3.68	3.42					
25387	42.7	9	5.85	2.43					
25848	40.0	8	6.50	2.48	24419		8	6.90	3.52
24672	34.0	7	7.06	2.95	24623	46.0	7	6.67	2.91

TABLE XXIII. CONCENTRATION OF REDUCING SUBSTANCES IN THE GASTROINTESTINAL TRACT OF STARVED CONTROL RATS OF SERIES III

Number of rat	Volume of washings	Wt. of reducing substances in 5 cc. aliquot	Wt. of reducing substances in gastrointestinal tract
	cc.	mg.	mg.
		Pregnant rats	
25416	250	0.28	13.77
25646	"	0.24	12.18
25967	"	0.21	10.40
25851	"	0.20	9.85
25799	"	0.23	11.34
Total Average		1.16 0.23	57.54 11.51
		Virgin rats	
25417	250	0.26	13.17
25648	"	0.26	12.82
25968	"	0.25	12.68
25852	"	0.26	12.82
25800	"	0.23	11.74
Total Average		1.26 0.25	63.23 12.65

TABLE XXIV. CONCENTRATIONS OF REDUCING SUBSTANCES IN THE GASTROINTESTINAL TRACT OF STARVED PORK-FED RATS OF SERIES III

Number of rat	Volume of washings	Wt. of reducing substances in 5 cc. aliquot	Wt. of reducing substances in gastrointestinal tract
	cc.	mg.	mg.
		Pregnant rats	
25388	250	---	10.34*
25420	"	0.26	12.82
25530	"	0.29	14.46
25645	"	0.27	13.72
25755	"	0.26	13.12
Total Average		1.08 0.27	54.12 13.53
		Virgin rats	
25389	250	---	11.47
25422	"	0.27	13.32
25531	"	0.23	11.74
25647	"	0.21	10.70
25756	"	0.20	10.10
Total Average		0.91 0.23	57.33 11.46

* Some of intestinal contents were lost. Omitted from average.

TABLE XXV. CONCENTRATION OF REDUCING SUBSTANCES IN THE GASTROINTESTINAL TRACT OF CONTROL RATS OF SERIES III AFTER FEEDING GLUCOSE

Number of rat	Volume of washings	Wt. of reducing substances in 5 cc. aliquot	Wt. of reducing substances in gastrointestinal tract
	cc.	mg.	mg.
		Pregnant rats	
25920	250	0.21	10.70
26080	"	0.19	9.70
26199	"	0.22	11.09
26270	"	0.22	11.09
26276	"	0.18	8.76
27927	"	0.20	9.95
28115	"	0.20	9.95
28291	"	0.20	10.65
Total Average		1.62 0.20	81.89 10.25
		Virgin rats	
25921	"	0.20	10.24
26081	"	0.24	11.88
26200	"	0.25	12.68
26271	"	0.18	8.96
27929	"	0.24	11.78
28336	"	0.36	18.08
28117	"	0.28	14.06
28293	"	0.28	14.02
Total Average		2.03 0.25	101.70 12.71

TABLE XXVI. CONCENTRATION OF REDUCING SUBSTANCES IN THE GASTROINTESTINAL TRACT OF PORK-FED RATS OF SERIES III AFTER FEEDING GLUCOSE

Number of rat	Volume of washings	Wt. of reducing substances in 5 cc. aliquot	Wt. of reducing substances in gastrointestinal tract
	cc.	mg.	mg.
		Pregnant rats	
25849	250	0.26	12.82
25918	"	0.25	12.48
25797	"	0.25	12.68
26116	"	0.25	12.38
26209	"	0.22	10.99
28290	"	0.27	13.42
28114	"	0.23	11.44
28489	"	0.27	13.67
Total Average		2.00 0.25	99.88 12.48
		Virgin rats	
25850	250	0.49	24.53
25919	"	0.22	10.89
25798	"	0.20	9.80
26117	"	0.21	10.30
26210	"	0.23	11.64
28292	"	0.26	12.92
28116	"	0.22	10.94
27928	"	0.18	9.05
Total Average		2.01 0.25	100.07 12.50

TABLE XXVII. CONCENTRATION OF REDUCING SUBSTANCES IN THE GASTROINTESTINAL TRACT OF RATS OF SERIES IV

Number of rat	Volume of washings	Wt. of reducing substances in 5 cc. aliquot	Wt. of reducing substances in gastrointestinal tract
	cc.	mg.	mg.
		Pregnant rats	
28412	250	0.28	13.96
28575	"	0.27	13.57
28798	"	0.22	10.89
28788	"	0.21	10.74
28893	"	0.24	12.28
Total		1.22	61.44
Average		0.24	12.29

TABLE XXVIII. CONCENTRATION OF LIVER GLYCOGEN IN STARVED PREGNANT CONTROL RATS OF SERIES III

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti+ placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
25416	226	154	56.2	50.7	3.2842 2.4063	5.6905	1.38 1.33	0.04 0.06	2.71	0.05
25646	217	129	--	--	3.7272 2.9053	6.6325	1.73 1.51	0.05 0.05	3.24	0.05
25967	261	123	55.5	49.2	3.0034 3.8062	6.8096	1.40 1.38	0.02 0.04	2.78	0.04
25851	226	142	44.5	38.7	3.1701 3.0261	6.1962	1.33 1.37	0.05 0.04	2.70	0.04
25799	250	133	49.4	43.2	3.4950 3.7424	7.2374	1.39 1.53	0.04 0.04	2.92	0.04
Total	1180	681	205.6	181.8		32.5662			14.35	0.22
Av.	236.0	136.2	51.4	45.4		6.5132			2.87	0.04

TABLE XXIX. CONCENTRATION OF LIVER GLYCOGEN IN STARVED VIRGIN CONTROL RATS OF SERIES III

Number of rat	Wt. of rat	Age of rat	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	mg.		mg.	
25417	156	144	1.8082 2.7569	4.5651	8.32 10.35	0.46 0.38	18.67	0.41
25648	152	159	2.3013 2.0731	4.3744	1.96 1.94	0.08 0.09	3.90	0.09
25968	184	129	2.5551 2.4379	4.9930	1.55 1.60	0.06 0.06	3.16	0.06
25852	176	147	2.2672 2.7721	5.0393	6.91 8.27	0.30 0.30	15.18	0.30
25800	170	171	2.2071 2.2729	4.4800	2.02 1.92	0.92 0.84	3.94	0.09
Total	838	750		23.4518			44.85	0.95
Av.	167.6	150.0		4.6904			8.97	0.19

TABLE XXX. CONCENTRATION OF LIVER GLYCOGEN IN STARVED PREGNANT PORK-FED RATS OF SERIES III

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
25388	258	130	48.0	41.5	5.3273 3.1978	8.5251	1.47 1.27	0.03 0.04	2.74	0.03
25420	278	125	62.5	54.7	4.1551 3.3554	7.5105	1.38 1.33	0.03 0.04	2.71	0.04
25530	256	124	47.5	41.5	3.0670 5.4006	8.4676	1.40 1.53	0.04 0.03	2.93	0.03
25645	226	137	38.5	33.1	2.8318 3.4703	6.3021	1.52 1.39	0.05 0.04	2.92	0.05
25755	240	128	41.5	34.7	3.7710 3.6305	7.4015	1.35 1.30	0.04 0.04	2.65	0.04
Total	1258	644	238.0	205.5		38.2068			13.95	0.19
Av.	251.6	128.8	47.6	41.1		7.6414			2.79	0.04

TABLE XXXI. CONCENTRATION OF LIVER GLYCOGEN IN STARVED VIRGIN PORK-FED RATS OF SERIES III

Number of rat	Wt. of rat	Age of rat	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	mg.		mg.	
25389	176	130	2.3283 3.0764	5.4047	3.15 3.21	0.14 0.10	6.36	0.12
25422	188	125	2.4005 2.7265	5.1270	2.06 2.20	0.12 0.08	4.26	0.08
25531	189	124	2.8346 2.7425	5.5771	6.46 7.20	0.23 0.26	13.66	0.24
25647	160	137	2.1194 2.2958	4.4152	2.80 3.06	0.13 0.13	5.86	0.13
25756	172	132	2.4191 3.1518	5.5709	49.46 52.47	2.04 1.66	101.92*	1.83*
Total	885	648		26.0949			30.14	0.57
Av.	177	129.6		5.2190			7.54	0.14

TABLE XXXII. CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT CONTROL RATS OF SERIES III AFTER FEEDING GLUCOSE

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
25920	214	140	45.2	39.7	3.2722 2.8570	6.1292	14.28 17.29	0.44 0.60	51.57	0.32
26080	264	128	51.5	45.5	3.0602 3.8583	6.8985	11.84 10.74	0.39 0.28	22.58	0.33
26199	270	145	65.0	58.3	3.3292 3.9888	7.3180	18.63 17.95	0.56 0.45	36.78	0.50
26270	234	112	48.0	41.2	3.3394 3.1193	6.4587	6.28 6.25	0.19 0.20	12.53	0.19
26276	280	136	77.5	61.7	3.5169 3.5317	7.0486	59.70 28.20	1.70 0.80	87.90	1.25
27927	258	155	54.2	48.0	3.8609 3.2317	7.0926	25.68 23.29	0.66 0.72	48.97	0.69
28115	248	140	61.2	55.4	2.8997 3.5200	6.4197	15.66 19.15	0.54 0.54	34.81	0.54
28291	242	126	51.2	46.3	3.2970 3.6136	6.9106	45.96 40.94	1.39 1.13	86.90	1.26
Total	2010	1082	453.8	396.1		54.2759			362.04	5.28
Av.	251.2	135.2	56.7	49.5		6.7845			45.26	0.66

TABLE XXXIII. CONCENTRATION OF LIVER GLYCOGEN IN VIRGIN CONTROL RATS OF SERIES III AFTER FEEDING GLUCOSE

Number of rat	Wt. of rat	Age of rat	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	mg.		mg.	
25921	167	158	2.4867 2.8731	5.3598	39.06 43.71	1.57 1.52	82.78	1.54
26081	182	141	3.1132 2.5115	5.6247	22.83 15.11	0.73 0.60	37.94	0.67
26200	200	191	3.3826 2.7621	6.1447	27.94 32.90	0.82 1.19	60.84	0.99
26271	160	118	1.8232 2.2774	4.1006	17.68 14.69	0.97 0.64	32.37	0.79
27929	175	185	2.6202 2.6476	5.2678	15.56 20.94	0.59 0.79	36.50	0.69
28336	177	140	3.0352 2.3891	5.4243	37.91 34.00	1.25 1.42	71.91	1.32
28117	174	152	2.6252 2.8189	5.4441	41.04 43.42	1.56 1.54	84.46	1.55
28293	174	130	2.8732 2.7973	5.6705	29.50 22.97	1.03 0.82	52.47	0.92
Total	1409	1215		43.0365			459.27	8.47
Av.	176.1	151.9		5.3796			57.41	1.06

TABLE XXXIV. CONCENTRATION OF LIVER GLYCOGEN IN PREGNANT PORK-FED RATS OF SERIES III AFTER FEEDING GLUCOSE

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
25849	244	125	47.5	40.8	3.5611 3.5724	7.1355	23.58 18.89	0.66 0.53	42.47	0.60
25918	250	113	58.4	50.4	3.9199 4.1047	8.0246	31.99 29.13	0.82 0.71	61.12	0.76
25797	254	172	45.9	36.2	4.1873 3.3614	7.5487	23.01 25.44	0.55 0.76	48.45	0.64
26116	230	137	47.6	41.4	3.3246 3.0653	6.3899	8.21 7.57	0.25 0.25	15.78	0.25
26209	252	123	50.0	54.1	3.2673 4.2805	7.5477	20.99 18.42	0.64 0.43	39.31	0.52
28290	233	132	17.3	13.3	4.2063 3.3894	7.5956	37.27 30.51	0.89 0.90	67.78	0.89
28114	230	147	29.5	25.1	3.0687 3.2578	6.3265	26.24 26.19	0.86 0.80	52.43	0.83
28489	250	117	20.5	16.2	4.0840 3.6806	7.7646	27.48 19.94	0.67 0.54	47.42	0.61
Total	1943	1066	314.7	279.5		58.3311			374.76	5.10
Av.	242.9	133.2	39.3	34.9		7.2914			46.84	0.64

TABLE XXXV. CONCENTRATION OF LIVER GLYCOGEN IN VIRGIN PORK-FED RATS OF SERIES III AFTER FEEDING GLUCOSE

Number of rat	Wt. of rat	Age of rat	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	mg.		mg.	
25850	175	123	2.9199 2.9696	5.8895	22.92 48.86	0.78 1.64	71.78	1.22
25919	147	126	2.7443 2.2941	5.0384	41.46 39.43	1.51 1.72	80.89	1.60
25798	174	185	2.6410 2.7791	5.4201	53.73 54.60	2.03 1.96	108.33	2.00
26117	172	144	2.5860 2.3150	4.9010	22.97 21.27	0.89 0.92	44.24	0.90
26210	178	128	2.8552 2.5648	5.4200	25.73 26.96	0.90 1.05	51.82	0.96
28292	168	139	2.5213 2.6019	5.1232	54.78 53.64	2.17 2.06	108.42	2.12
28116	180	152	2.4997 2.7332	5.2329	43.33 43.48	1.73 1.59	86.81	1.66
27928	184	141	3.1941 2.5068	5.7009	50.05 43.24	1.57 1.72	93.29	1.64
Total	1378	1138		42.7260			645.58	12.10
Av.	172.2	142.2		5.3408			80.70	1.51

TABLE XXXVI. CONCENTRATION OF LIVER GLYCOGEN IN RATS OF SERIES IV

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti + placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
28412	244	115	43.8	37.3	3.4025 3.9562	7.3587	24.76 27.24	0.73 0.69	52.00	0.71
28575	238	96	45.8	43.4	3.0206 3.5244	6.5450	9.04 9.18	0.30 0.26	18.22	0.28
28798	235	118	28.3	24.1	3.5521 3.4072	6.9593	35.66 38.14	1.00 1.12	73.80	1.06
28788	276	138	60.4	53.2	3.4697 3.7927	7.2624	20.86 27.61	0.60 0.73	48.47	0.67
28893	228	129	--	--	4.1780 2.6345	6.8125	43.43 31.01	1.04 1.18	74.44	1.09
Total	1221	596	178.3	158.0		34.9379			266.93	3.81
Av.	244.2	119.2	44.6	39.5		6.9876			53.39	0.76

TABLE XXXVII. QUANTITY OF GLUCOSE ADMINISTERED TO PREGNANT CONTROL RATS OF SERIES III

Number of rat	Dilution factor		Wt. of glucose in 5 cc. aliquct		Wt. of glucose in washings from catheter	Wt. of glucose in solution administered plus washings	Wt. of glucose administered
	Washings	Glucose solution	Washings	Glucose solution			
			mg.	mg.	mg.	mg.	mg.
25920	200	1000	1.3001	1.0372	260.02	1037.20	777.18
26080	"	"	1.3179	1.0987	263.58	1098.70	835.12
26199	"	"	1.7266	1.1453	345.32	1145.30	799.98
26270	"	"	1.7167	1.0818	343.34	1081.80	738.46
26276	"	"	1.6959	0.8944	339.18	894.40	555.22
27927	"	"	1.9627	1.0957	392.54	1095.70	703.26
28115	"	"	1.8873	1.1116	377.46	1111.61	734.15
28291	"	"	1.8715	1.1393	374.30	1139.30	765.00
Total					2695.74	8604.01	5908.37
Av.					336.97	1075.50	738.55

TABLE XXXVIII. QUANTITY OF GLUCOSE ADMINISTERED TO VIRGIN CONTROL RATS OF SERIES III

Number of rat	Dilution factor		Wt. of glucose in 5 cc. aliquot		Wt. of glucose in washings from catheter	Wt. of glucose in solution administered plus washings	Wt. of glucose administered
	Washings	Glucose solution	Washings	Glucose solution			
			mg.	mg.	mg.	mg.	mg.
25921	200	1000	1.7742	1.1086	354.84	1108.60	753.76
26081	"	"	1.7573	1.1324	351.46	1132.40	780.94
26200	"	"	1.3050	1.1176	261.00	1117.50	856.50
26271	"	"	1.5808	1.0957	316.16	1095.70	779.54
27929	500	"	0.8507	1.1175	425.35	1117.50	692.15
28336	"	"	0.6186	1.0897	309.30	1089.70	780.40
28117	"	"	0.6126	1.1066	306.30	1106.64	800.34
28293	"	"	0.8388	1.1175	419.40	1117.50	698.10
Total					2743.81	8885.54	6141.73
Av.					342.98	1110.69	767.72

TABLE XXXIX. QUANTITY OF GLUCOSE ADMINISTERED TO PREGNANT FORK-FED RATS OF SERIES III

Number of rat	Dilution factor		Wt. of glucose in 5 cc. aliquot		Wt. of glucose in washings from catheter	Wt. of glucose in solution administered plus washings	Wt. of glucose administered
	Washings	Glucose solution	Washings	Glucose solution			
			mg.	mg.	mg.	mg.	mg.
25849	---	1000		0.8765	-----	---	876.50
25918	200	"		0.8725	-----	---	872.50
25797	"	"	1.3507	1.1106	270.14	1110.60	840.46
26116	"	"	1.7742	1.1433	354.84	1143.30	788.46
26209	"	"	1.6750	1.0739	335.00	1073.91	738.91
28290	"	"	1.9151	1.1493	383.02	1149.30	766.28
28114	500	"	0.8279	1.0917	413.95	1091.70	677.75
28489	"	"	0.6474	1.1046	323.70	1104.66	780.66
Total					2080.65	6673.47	6341.82
Average					346.78	1112.24	792.73

TABLE XL. QUANTITY OF GLUCOSE ADMINISTERED TO VIRGIN PORK-FED RATS OF SERIES III

Number of rat	Dilution factor		Wt. of glucose in 5 cc. aliquot		Wt. of glucose in washings from catheter	Wt. of glucose in solution administered plus washings	Wt. of glucose administered
	Washings	Glucose solution	Washings	Glucose solution			
			mg.	mg.	mg.	mg.	mg.
25850	---	1000	---	0.8854	---	---	885.40
25919	200	"	1.3973	1.1195	279.46	1119.50	840.04
25798	"	"	1.6870	1.1096	337.40	1109.60	772.20
26117	"	"	1.5580	1.0540	311.60	1054.00	742.40
26210	"	"	1.6750	1.0739	337.00	1095.70	758.70
28292	500	"	0.6464	1.1592	323.20	1159.20	836.00
28116.	"	"	0.5928	1.1066	296.40	1106.60	810.20
27928	200	"	1.3636	1.1116	272.72	1111.60	838.88
Total					2157.78	7756.20	6483.82
Av.					308.25	1108.03	810.48

TABLE XLI. QUANTITY OF GLUCOSE ADMINISTERED TO RATS OF SERIES IV

Number of rat	Dilution factor		Wt. of glucose in 5 cc. aliquot		Wt. of glucose in washings from catheter	Wt. of glucose in solution administered plus washings	Wt. of glucose administered
	Washings	Glucose solution	Washings	Glucose solution			
			mg.	mg.	mg.	mg.	mg.
28412	500	1000	0.9231	1.2217	461.55	1221.70	760.15
28575	"	"	0.6503	1.1235	325.15	1123.50	798.35
28798	"	"	0.6523	1.1195	326.15	1119.50	793.35
28788	"	"	0.6811	1.2197	340.55	1219.70	879.15
28893	"	"	0.6969	1.0094	348.45	1009.40	660.95
Total					1801.85	5693.80	3891.95
Av.					360.37	1138.76	778.39

TABLE XLII. QUANTITY OF GLUCOSE ABSORBED BY PREGNANT CONTROL RATS OF SERIES III

Number of rat	Wt. of rat	Wt. of glucose administered	Wt. of reducing substances in gastrointestinal tract	Av. wt. of reducing substances in gastrointestinal tract of starved rat	Wt. of glucose absorbed	Per cent glucose absorbed
	gm.	mg.	mg.	mg.	mg.	
25920	214	777.18	10.70	11.51	777.99	100.10
26080	264	835.12	9.70	"	836.93	100.22
26199	270	799.98	11.09	"	800.40	100.05
26270	234	738.46	11.09	"	738.88	100.06
26276	280	555.22	8.76	"	557.97	100.50
27927	258	703.26	9.95	"	704.82	100.22
28115	248	734.15	9.95	"	735.71	100.21
28291	242	765.00	10.65	"	765.86	100.11
Total	2010	5908.37	81.89		5918.56	801.47
Av.	251.2	738.55	10.25		739.82	100.18

TABLE XLIII. QUANTITY OF GLUCOSE ABSORBED BY VIRGIN CONTROL RATS OF SERIES III

Number of rat	Wt. of rat	Wt. of glucose administered	Wt. of reducing substances in gastrointestinal tract	Av. wt. of reducing substances in gastrointestinal tract of starved rat	Wt. of glucose absorbed	Per cent glucose absorbed
	gm.	mg.	mg.	mg.	mg.	
25921	167	753.76	10.24	12.65	756.17	100.32
26081	182	780.94	11.88	"	781.71	100.10
26200	200	856.50	12.68	"	856.47	100.00
26271	160	779.54	8.96	"	783.23	100.73
27929	175	692.15	11.78	"	693.02	100.12
28336	177	780.40	18.08	"	774.97	99.30
28117	174	800.34	14.06	"	798.93	99.82
28293	174	698.10	14.02	"	696.73	99.80
Total	1409	6141.73	101.70		6141.23	800.19
Av.	176.1	767.72	12.71		767.65	100.02

TABLE XLIV. QUANTITY OF GLUCOSE ABSORBED BY PREGNANT PORK-FED RATS OF SERIES III

Number of rat	Wt. of rat	Wt. of glucose administered	Wt. of reducing substances in gastrointestinal tract	Av. wt. of reducing substances in gastrointestinal tract of starved rat	Wt. of glucose absorbed	Per cent glucose absorbed
	gm.	mg.	mg.	mg.	mg.	
25849	244	876.50	12.82	13.53	877.21	100.08
25918	250	872.50	12.48	"	873.55	100.12
25797	254	840.46	12.68	"	841.31	100.10
26116	230	788.46	12.38	"	789.61	100.14
26209	252	738.91	10.99	"	741.45	100.34
28290	233	766.28	13.42	"	766.39	100.01
28114	230	677.75	11.44	"	679.84	100.31
28489	250	780.96	13.67	"	780.82	99.98
Total	1943	6341.81	99.88		6350.18	801.08
Av.	242.9	792.73	12.48		793.77	100.14

TABLE XLV. QUANTITY OF GLUCOSE ABSORBED BY VIRGIN PORK-FED RATS OF SERIES III

Number of rat	Wt. of rat	Wt. of glucose administered	Wt. of reducing substances in gastrointestinal tract	Av. wt. of reducing substances in gastrointestinal tract of starved rat	Wt. of glucose absorbed	Per cent glucose absorbed
	gm.	mg.	mg.	mg.	mg.	
25850	175	885.40	24.53	11.46	872.33	98.52
25919	147	840.04	10.89	"	840.61	100.07
25798	174	772.20	9.80	"	773.86	100.21
26117	172	742.40	10.30	"	743.56	100.16
26210	178	758.70	11.64	"	758.52	99.98
28292	168	836.00	12.92	"	834.54	99.82
28116	180	810.20	10.94	"	810.72	100.06
27928	184	838.88	9.05	"	841.29	100.29
Total	1378	6483.82	100.07		6475.43	799.11
Av.	172.2	810.48	12.50		809.43	99.89

TABLE XLVI. QUANTITY OF GLUCOSE ABSORBED BY RATS OF SERIES IV

Number of rat	Wt. of rat	Wt. of glucose administered	Wt. of reducing substances in gastrointestinal tract	Av. wt. of reducing substances in gastrointestinal tract of starved rat	Wt. of glucose absorbed	Per cent glucose absorbed
	gm.	mg.	mg.	mg.	mg.	
28412	244	760.15	13.96	11.46	757.65	99.67
28575	238	798.35	13.57	"	796.24	99.74
28798	235	793.35	10.89	"	793.92	100.07
28788	276	879.15	10.74	"	879.87	100.08
28893	228	660.95	12.28	"	661.77	100.12
Total	1221	3891.95	61.44		3869.45	499.68
Av.	244.2	778.39	12.29		777.89	99.94

TABLE LXVII. LIVER GLYCOGEN DEPOSITION IN PREGNANT CONTROL RATS OF SERIES III IN TERMS OF GLYCOGEN INDEX

Number of rat	Wt. of rat	Mg. of glucose absorbed	Mg. of liver glycogen per 100 gm. liver	Mg. glucose absorbed per 100 gm. of rat	Glycogen index
	gm.				
25920	214	777.99	520	363.55	1.43
26080	264	836.93	330	317.02	1.04
26199	270	800.40	500	296.44	1.69
26270	234	738.88	190	315.76	0.60
26276	280	557.97	1250	199.28	6.27*
27927	258	704.82	690	273.19	2.52
28195	248	735.71	540	296.66	1.82
28291	242	765.86	1260	316.47	3.98
Total	2010				13.03
Av.	251				1.86

* Omitted from average

TABLE LXVIII. LIVER GLYCOGEN DEPOSITION IN VIRGIN CONTROL RATS OF SERIES III IN TERMS OF GLYCOGEN INDEX

Number of rat	Wt. of rat	Mg. of glucose absorbed	Mg. of liver glycogen per 100 gm. liver	Mg. glucose absorbed per 100 gm. of rat	Glycogen index
	gm.				
25921	167	756.17	1540	452.80	3.40
26081	182	781.71	670	429.51	1.56
26200	200	856.47	990	428.24	2.31
26271	160	783.23	790	489.52	1.61
27929	175	693.02	690	396.01	1.74
28336	177	774.97	1320	437.84	3.01
28117	174	798.93	1550	459.16	3.38
28293	174	696.73	920	400.42	2.30
Total	1409				19.31
Av.	176.1				2.41

TABLE XLIX. LIVER GLYCOGEN DEPOSITION IN PREGNANT PORK-FED RATS OF SERIES III IN TERMS OF GLYCOGEN INDEX

Number of rat	Wt. of rat	Mg. of glucose absorbed	Mg. of liver glycogen per 100 gm. liver	Mg. glucose absorbed per 100 gm. of rat	Glycogen index
	gm.				
25849	244	877.21	600	359.51	1.67
25918	250	873.55	760	349.42	2.18
25797	254	841.31	640	331.22	1.93
26116	230	789.61	250	343.31	0.73
26209	252	741.45	520	294.22	1.77
28290	233	766.39	890	328.92	2.70
28114	230	679.84	830	295.58	2.81
28489	250	780.82	610	312.33	1.95
Total	1943	6350.18		2614.51	15.74
Av.	242.9	793.77		326.81	1.97

TABLE L. LIVER GLYCOGEN DEPOSITION IN VIRGIN PORK-FED RATS OF SERIES III IN TERMS OF GLYCOGEN INDEX

Number of rat	Wt. of rat	Mg. of glucose absorbed	Mg. of liver glycogen per 100 gm. liver	Mg. glucose absorbed per 100 gm. of rat	Glycogen index
	gm.				
25850	175	872.33	1220	498.47	2.45
25919	147	840.61	1600	571.84	2.80
25798	174	773.86	2000	444.75	4.50
26117	172	743.56	900	432.30	2.08
26210	178	758.52	960	426.13	2.25
28292	168	834.54	2120	496.75	4.27
28116	180	810.72	1660	450.40	3.68
27928	184	841.29	1640	457.22	3.59
Total	1378			3777.86	25.62
Av.	172.2			472.23	3.20

TABLE LI. LIVER GLYCOGEN DEPOSITION IN RATS OF SERIES IV IN TERMS OF GLYCOGEN INDEX

Number of rat	Wt. of rat	Mg. of glucose absorbed	Mg. of liver glycogen per 100 gm. liver	Mg. glucose absorbed per 100 gm. of rat	Glycogen index
	gm.				
28412	244	757.65	710	310.51	2.29
28575	238	796.24	280	334.55	0.84
28798	235	793.92	1060	337.84	3.14
28788	276	879.87	670	318.79	2.10
28893	228	661.77	1090	290.25	3.76
Total	1221	3889.45	3810	1591.94	12.13
Av.	244.2	777.89	760	318.39	2.43

TABLE LII. CONCENTRATION OF LIVER GLYCOGEN IN RATS OF SERIES V

Number of rat	Wt. of rat	Age of rat	Wt. of intact uterus	Wt. of feti+ placentae	Wt. of liver sample	Total wt. of liver	Wt. of glycogen in liver sample	Per cent glycogen in liver sample	Wt. of glycogen in liver	Per cent glycogen in liver
	gm.	days	gm.	gm.	gm.	gm.	mg.		mg.	
28701	145	86	--	--	2.2731 2.3161	4.5892	17.87 20.62	0.79 0.89	38.49	0.84
27984	198	84	44.2	39.7	2.7151 3.3338	6.0489	0.00 0.00	0.00	0.00 0.00	0.00
28709	193	87	--	--	3.6705 3.1906	6.8611	78.74 67.84	2.14 2.13	146.59	2.14
24399	234	67	53.5	43.5	2.8234 3.8730	6.6964	0.00 0.00	0.00	0.00 0.00	0.00

FORM I. CONDITION OF FEMALE RATS AT AUTOPSY (EXTERNAL CONDITION)

Rat no. _____ Diet no. _____ Description of diet _____
Age rat (in days) _____ Autopsy date _____ Post-starvation period
Pregnancy no. _____ Hour _____ Food _____
Day of gestation period _____ Period of starvation _____ Quantity _____
Wt. before starving _____ Hour initiated _____ Length of period _____
Wt. after starving _____ Hour terminated _____

Physical condition[1]
General _____ Alert _____ Paws pinkish _____
Fat _____ Gaunt _____ Eyes pink _____

Eyelids
Inflamed _____ Infected _____

Muscle tone
General _____ Abdominal _____

Respiration
Sniffy _____ Wheezy _____
Palpitations _____

Gait
Dragging _____ Elevated _____
Sprawling _____ Awkward _____

Exudates[2]
Nasal _____ Anal _____
Oral _____ Vaginal _____

Hair
Clean _____ Smooth _____
Creamy _____ Thick _____
Fine _____

Hematuria _____

Remarks _____

Tail
Clean _____ Smooth _____
Discolored _____ Sores _____

1. In recording the degree to which any condition is present use a scale ranging from minus (-) to four plusses (++++).
2. Indicate character of exudate

FORM II. CONDITION OF FEMALE RATS AT AUTOPSY (INTERNAL CONDITION)

Rat no. _____ Diet no. _____ Description of diet _____

Fat depots[1]
Subcutaneous _____ Perirenal _____
Peritoneal _____ Genital _____
Omental _____ Intermuscular _____

Liver
Yellow _____ Mottled _____
Friable _____ Spongy _____

_____

Kidneys
Cortex, color _____ friable _____
Medulla, color _____ friable _____
Pelvis, color _____ friable _____

_____

_____

Pancreas, any gross abnormalities: _____

Corpora lutea
No. in left ovary _____ right _____
Color _____

Fetal sites, no. of _____

Live feti, no. of _____

Resorptions, no. of _____

Stomach ulcers
Number _____ Severity _____

Condition of the lungs:

	Infection	Atelectosis	Emphysema
Lobe 1.			
2.			
3.			
4.			
5.			

Pus pockets:
Ovary _____
Placental sites _____
Ear _____
Base of the tongue _____

Teeth
Straight _____ Orange _____

Remarks _____

_____

1. Use a scale ranging from minus (-) to four plusses (++++) in so far as possible in recording the degree to which any condition is present.